The peri-implant soft tissue reactions and cytokine expressions around different abutment materials : randomized controlled clinical trial

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การทดลองแบบสุ่มเพื่อเปรียบเทียบการตอบสนองของเนื้อเยื่อรอบรากเทียมและการแสดงออกของไซโตไคน์บนวัสดุฐานรองครอบฟันบนรากเทียมแต่ละชนิด

น.ส.ประภาพรรณ ใจกล้า

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ประภาพรรณ ใจกล้า : การทดลองแบบสุ่มเพื่อเปรียบเทียบการตอบสนองของเนื้อเยื่อรอบรากเทียมและการแสดงออกของไซโตไคน์บนวัสดุฐานรองครอบฟันบนรากเทียมแต่ละชนิด. (The peri-implant soft tissue reactions and cytokine expressions around different abutment materials : randomized controlled clinical trial) อ.ที่ปรึกษาหลัก : รศ.ประเวศ เสรีเชษฐพงษ์, อ.ที่ปรึกษาร่วม : รศ. ดร.อาทิพันธุ์ พิมพ์ขาวขำ

วัตถุประสงค์ เพื่อเปรียบเทียบความแตกต่างของปฏิกิริยาภูมิคุ้มกันของเนื้อเยื่อรอบรากเทียมและการแสดงออกของไซโตไคน์โดยศึกษาจากสารคัดหลั่งในร่องปริทันต์ของวัสดุฐานรองครอบฟันบนรากเทียมแต่ละชนิด คือ ไทเทเนียม เซอร์โคเนีย และโลหะผสมทอง

วิธีการศึกษาวิจัย รากเทียมในบริเวณฟันหลังทั้งหมด 15 ตัว แบ่งออกเป็น 3 กลุ่มอย่างสุ่มและใส่ฐานรองครอบฟันบนรากเทียมภายในวันเดียวกันกับการผ่าตัดฝังรากเทียม เมื่อครบกำหนดสัปดาห์ที่ 4, 6, 8 และ 10 สารคัดหลั่งในร่องปริทันต์, ดัชนีคราบจุลินทรีย์และดัชนีค่าเหงือกอักเสบจะถูกเก็บเพื่อการประเมินผลเนื้อเยื่อรอบรากเทียม ระดับของไซโตไคน์จากสารคัดหลั่งในร่องปริทันต์ของวัสดุฐานรองครอบฟันแต่ละชนิดถูกนำไปผ่านกระบวนการใช้ปฏิกิริยาที่เฉพาะเจาะจงของแอนติบอดีและแอนติเจนโดยใช้เอนไซม์ การวิเคราะห์ข้อมูลโดยวิธีสถิติnon-parametric

ผลการวิจัย วัสดุฐานรองครอบฟันบนรากเทียมชนิดโลหะผสมทองแสดงผลของระดับไซโตไคน์ชนิด IL-1beta และ IL-6 สูงกว่าไทเทเนียมและเซอร์โคเนียที่สัปดาห์ 4, 6 และ 8 แต่ไม่แสดงผลแตกต่างของระดับไซโตไคน์ชนิด IL-8 ในทุกสัปดาห์ เมื่อเปรียบเทียบระหว่างกลุ่มของการแสดงผลระดับไซโตไคน์ชนิด IL-1beta สัปดาห์ที่ 4 และ 6 พบว่าโลหะผสมทองแตกต่างจากเซอร์โคเนียอย่างมีนัยสำคัญ (p-value 0.024 และ 0.032 ตามลำดับ) โลหะผสมทองและไทเทเนียมที่สัปดาห์ 4, 6 และ 8 แตกต่างอย่างมีนัยสำคัญของระดับไซโตไคน์ชนิด IL-1beta (p-value 0.015, 0.022 และ 0.033 ตามลำดับ) ค่าความหยาบของผิววัสดุแต่ละชนิดแสดงผลไม่แตกต่างกัน อย่างไรก็ตามดัชนีคราบจุลินทรีย์และดัชนีค่าเหงือกอักเสบแสดงผลแตกต่างกันในแต่ละวัสดุ ผลการทดลองสนับสนุนว่าวัสดุแต่ละชนิดส่งผลต่อการตอบสนองภูมิคุ้มกัน

สรุปผลการวิจัย วัสดุฐานรองครอบฟันแต่ละชนิดส่งผลต่อการแสดงออกภูมิคุ้มกันและลักษณะเนื้อเยื่อรอบรากเทียม โดยโลหะผสมทองแสดงผลของระดับไซโตไคน์มากกว่าไทเทเนียมและเซอร์โคเนียร่วมกับการมีค่าดัชนีคราบจุลินทรีย์และดัชนีเหงือกอักเสบสูงกว่า ดังนั้นควรให้ทันตสุขศึกษาในผู้ป่วยที่ใช้วัสดุฐานรองครอบฟันชนิดโลหะผสมทองโดยเฉพาะในช่วงแรกของการหายของแผล

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KEYWORD: Enzyme-linked immunosorbent assay (ELISA), peri-implant crevicular fluid, cytokine level, dental implant abutment

Prapaphan Jaikla : The peri-implant soft tissue reactions and cytokine expressions around different abutment materials : randomized controlled clinical trial. Advisor: Assoc. Prof. PRAVEJ SERICHETAPHONGSE Co-advisor: Assoc. Prof. ATIPHAN PIMKHAOKHAM, Ph.D.

Objectives : Stable peri-implant soft tissue around transmucosal zone are the crucial factor for long-term success and survival of dental implants. The aim of this study was to evaluate the expression of proinflammatory cytokines and chemokine around 3 types of abutment materials : titanium (Ti), zirconium oxide (Zr) and gold alloy (Au).

Methodology : 15 dental implants were enrolled in this study. Clinical parameters and peri-implant crevicular fluid (PICF) were collected at weeks 4, 6, 8 and 10. The soft tissue characteristics were demonstrated using plaque assessment score and mucosal condition score. Cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA). Nonparametric statistics were used to describe the comparison of abutment materials and cytokine levels.

Results and Discussion : At 4,6 and 8-week of healing period, gold alloy abutments induced the highest level of IL-1beta and IL-6. In pairwise test, there were significant differences in IL-1beta at week 4 and 6 between Au and Zr abutment p-value 0.024 and 0.032, respectively. For Au and Ti abutment, statistical significances were observed at week 4, 6 and 8 p-value 0.015, 0.022 and 0.033, respectively. The analyses compared values of weeks 4, 6, 8 and 10 showed there were no significant differences in IL-8 between abutment materials. The average surface roughness of abutment material was reported similar roughness. However, different materials exhibited different plaque and mucosal condition score. These findings supported the implant abutment materials have an influence on the immune response.

Conclusion : Gold alloy abutment induced higher levels of IL-1beta and IL-6 in PICF when compared with titanium and zirconium oxide abutment at weeks 4. 6 and 8 whereas no significant differences in the expression of IL-8 all time points. Higher plaque score and mucosal tissue conditions were reported in gold alloy abutment. Therefore, strict oral hygeine instructions should be given to patients when using gold alloy abutment especially in early healing period.

Field of Study:	Esthetic Restorative and Implant Dentistry	Student's Signature .....
Academic Year:	2018	Advisor's Signature .....
		Co-advisor's Signature .....

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Prapaphan Jaikla

TABLE OF CONTENTS

LIST OF TABLES

page

LIST OF FIGURES

Page

BACKGROUND AND RATIONALE

The success and sustainability of dental implant depends upon both mechanical effect and biological effect of dental implant toward hard and soft tissue.(1-3) Dental implant must be placed in proper position and angulation to gain thickness of labial bone and soft tissue. Utilizing a suitable implant abutment, esthetic outcome can be achieved.(4, 5) Consequently, the sustainable of the pleasing appearance depends on tissue responses. Soft tissue attachment with keratinized mucosa in the transmucosal zone at dental abutment level serve as so called "a biological cuff" which is an essential parameter for preventing microbial invasion. The collagen fibers of the connective tissue around dental implant arrange themselves both parallel and circular to the implant abutment surface. Unlike Sharpey fibers in a natural tooth which penetrate toward cementum or "biological seal", the junctional epithelium of the peri-implant mucosa is attached to the titanium abutment surface with hemidesmosomes at basal lamina.(6-9) Because of a vulnerable gingival architecture around implant abutment, an abutment material used that can provoke any inflammation reaction of soft tissue must be avoid. So far there are very few prospective human studies reported in this matter.

The cardinal signs of inflammation of soft tissue includes redness, edematous consistency (swelling), pain and bleeding on probing. If these clinical features appear, hard and soft tissue irreversible destruction occur. A recent human study reported with histological section of gingiva around dental implant abutment showed differences in the amount of inflammatory cells in various abutment materials used.(10) Other histo-immunological studies also presented with different responses of soft tissue toward different implant abutment materials. However, most of the studies were done in animal model.(6, 7, 11, 12)

Currently, the evaluation of such cytokines in human model utilizing the peri-implant crevicular fluid (PICF) has been proposed as a noninvasive means of monitoring the healthy or diseased status of peri-implant tissue. (13) Biological mediators (e.g., cytokines, chemokine and bone markers) released by cells of the peri-implant mucosa can be used to characterize the responses of given abutment materials. (14)

REVIEW OF LITERATURE

Since the osseointegrated implants in the treatment of the edentulous patient have been described in 1981, the implant-supported fix prostheses become the effective treatment of choice in oral rehabilitation. In modern dentistry the dental implants have been widely used in order to restore functions, comfort, speech, esthetics and health in partially or fully-edentulous patients due to its high survival and success rate.(15-17)

The crestal bone stability and healthy soft tissue are the important factor for long-term success of dental implant.(1) Soft tissue attachment around dental abutments has some similarities to that of natural teeth including mucosa, junctional epithelium and connective tissue attachments. However, there are some differences between the connective tissue attachments around implants and teeth. In order to create biologic bond around natural teeth, the Sharpey fibers are oriented and inserted in cementum which makes the cementum an essential functional part of periodontium. On the other hand, the collagen fibers of the connective tissue around dental implant run parallel and circular to the abutment surface known as a cuff-like barrier. The junctional epithelium attaches the abutment surface via

hemidesmosomes at basal lamina. The peri-implant soft tissue attachment in the transmucosal zone of dental abutments serves as a biological seal which is an essential parameter for preventing microbial invasion. In addition, the peri-implant connective tissue shows poor vascularity and appears more like a scar tissue.(7, 11, 18, 19) (Fig 1, Table 1)

Table 1 Natural tooth VS. Dental implant (19)

Parameter	Natural Tooth	Dental Implant
Biological width	2.04 to 2.91 mm	3.08 mm
Mean connective tissue width	1.12 mm	1.66 mm
Type of junctional epithelium attachment	Hemidesmosomes	Partially hemidesmosomes
Connective tissue attachment	Perpendicular to the cementum (Sharpey fibers)	Layer of proteoglycans, 20 μm thick
Collagen fiber insertion	Thirteen groups: perpendicular to tooth surfaces	Two groups: parallel and circular fibers (as scar tissue)

Ratio of collagen fibers to fibroblasts	60% collagen fibers to 5-15% fibroblasts	85% collagen fibers to 1-3% fibroblasts
Vascularity	Greater Supraperiosteal and periodontal ligament	Less Supraperiosteal

There are several factors influencing the transmucosal zone such as the surface topography, surface energy and chemical characteristics of dental abutment as well as the prosthetic components and connections. Abutments are considered one of the most important components of implant-supported restoration because they establish the connection between the intraosseous structure and the prosthetic part. It is crucial to control inflammation around dental implants to maintain the health of adjacent soft tissues, to decrease bone resorption and to increase the longevity of implants.(3, 5, 20) Therefore, the requisites to the long-term stability of an osseointegrated implant is the use of optimal biomechanical and biocompatible characteristics of dental implant abutment. (21, 22)

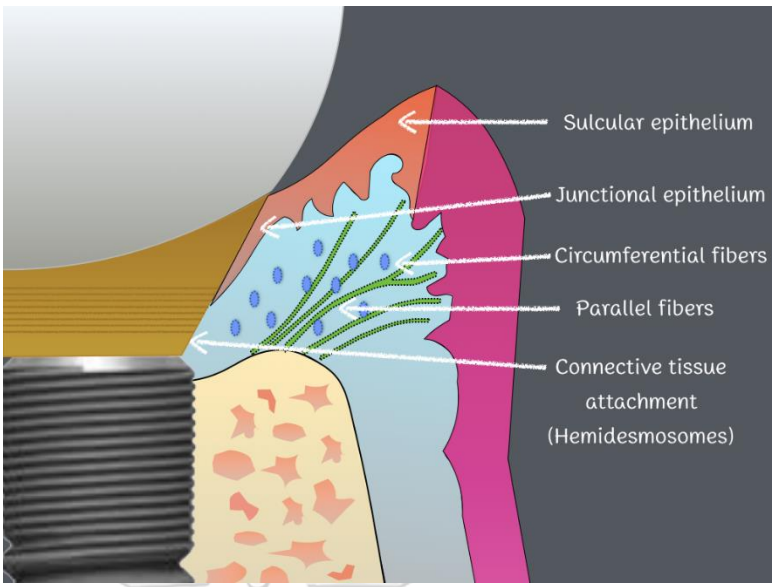


Figure 1 Peri-implant soft tissue zone around dental abutment

ABUTMENT MATERIAL

Dental implant abutment is a critical part of implant treatment as a transmucosal component because they exhibit the relationship between the intraosseous structure (implant fixture) and the prostheses. Abrahamsson et al claim that abutment materials may play important roles in the prevention of crestal bone and soft tissue recession.(7) A variety of implant abutments differing in design and biomaterials have been introduced to achieve optimal mechanical, biological and esthetic outcomes. Long-term clinical studies on commercially pure titanium demonstrated excellent survival rates for fixed implant reconstructions.(3) In a recent systemic review, only a

few complications were associated with metal abutments supporting fixed implant reconstructions. Therefore, titanium abutment represents the gold standard for implant restoration. However, the major drawback of titanium abutment is dark grey color. It may shine through the peri-implant soft tissue and cause esthetic problems. Another type of metal abutment materials has been widely used since 1988 is a customized cast metal that introduced by Beumer et al. The University of California Los Angeles known as UCLA abutment are the first customized cast metal component to be directly screwed into the dental implant.(23, 24) The yellow color of gold can enhance the pink color of gingiva which results in favorable esthetic outcome.(20) Nevertheless, the animal studies have been shown that no proper mucosal seal around gold abutment. As a result of less biocompatibility and higher pricing, their use has been decreasing.(10)

In order to solve these problems, producing a densely sintered alumina ceramic abutment was introduced in 1993 by mean of computer-aided design – computer-aided manufacturing (CAD-CAM) technology. Then, Glauser et al. described the yttrium-stabilized zirconia as an alternative ceramic abutment.(4) To date, milling technology facilitates precise component fabrication from

durable and esthetic materials. It is important for anterior region because peri-implant soft and hard tissue morphology have direct influence on esthetic outcomes and stability of implant placement. The proper selection of anatomical shaped implant abutment can help creating the proper emergence profile and supporting peri-implant soft tissue. Zirconia abutment offers a better esthetic outcome superior to titanium abutment especially in thinner peri-implant mucosa or patients with high or gummy smiles.(25) Besides the favorable color appearance, zirconia abutments have been shown in several studies with less initial plaque accumulation than titanium abutment.(26, 27) According to a systematic reviewed by Linkevicius and Aspe in 2008, animal histologic studies showed the reaction of peri-implant soft and hard tissues in titanium similar to zirconia.(5) In addition to human histological studies, zirconia has a better reaction of peri-implant mucosa compared to titanium. This has also been confirmed in clinical studies. Randomized controlled clinical trial were conducted by Sailer and Zembic in 2009 to test the survival and technical and biological outcome between zirconia and titanium abutment. At one and three years in functions, zirconia abutment showed similarly in survival and technical, biological and esthetic outcomes as titanium abutment.(28, 29) There were no significant

differences in biologic and radiographic parameters as well as marginal bone loss after five years of function in posterior regions.(30) However, the mechanical properties of ceramic abutments that are brittle may be a shortcoming and prone to fatigue. Among all fractures, the highest fractures were reported for alumina abutments followed by zirconia abutment. There were no reports the fractures on titanium and cast metal alloy abutment for anterior region.(31) Recently, in vitro study showed wear of titanium platform in direct contact with zirconia abutment. The implant surface deterioration and the accumulation of titanium wear particles may affect osseointegration and health of peri-implant tissue. Therefore, the different mechanical properties of the titanium implant and zirconia abutment have to be concerned at the implant-abutment interface.(32)

GINGIVAL CREVICULAR FLUID

The gingival sulcus is an area between the marginal gingiva and the enamel or cementum. It is bounded by the tooth surface on one side, the sulcular epithelium on the other side, and the junctional epithelium as its most apical point. The crevice or the pocket is bathed by the gingival crevicular fluid, which carries the soluble immunological contents.(33) Gingival crevicular fluid

is defined as a serum transudate or an inflammatory exudate of the periodontal tissues, in health and disease, respectively. Exudate is the result of an increase in the permeability of the vessels underlying junctional and sulcular epithelium, allowing plasma to leak into the crevice. Moreover, an exudate contains higher content of protein, including major plasma proteins and immunologically active components.(34) The gingival crevicular fluid acts as a medium for the carriage and transport of various bacterial products into the gingiva, or host-derived immune components outwards.(33) The cellular components of crevicular fluid include exfoliated epithelial cells from junctional or sulcular epithelium, bacteria from biofilm on the tooth surface and cells migrating from the blood circulation.(35, 36) The molecular elements of the gingival crevicular fluid include host enzymes, immunoglobulins, complement proteins, inflammatory mediators, tissue degradation products, cell-lysis components, as well as bacterial metabolic and lysis products.(33, 37) The numerous biomarkers in gingival crevicular fluid, proinflammatory cytokines [e.g. TNFα, IFNγ, IL-1, IL-6, IL-12, IL-17 and RANKL], anti-inflammatory cytokines [e.g. IL-4, IL-10 and IL-1ra] and chemokines [e.g. IL-8] have been suggested to be important mediators of inflammation. Local balance of these mediators that reflects local activity

of cells that produce them, determines the level of tissues destruction.(13, 37, 38) Eventually, the gingival crevicular fluid is a good reflection of the inflammatory state of the tissue. Because of a technical advantage of gingival crevicular fluid, it can be easily and noninvasively collected from the periodontal pocket. Most of the published studies used the analysis of the immunological content of gingival crevicular fluid as a diagnostic tool for periodontal disease.(13, 33, 34, 39) Protein immunoassays which are biochemical or antibody-based methods have been used extensively for the characterization of gingival crevicular fluid, such as immunoblotting or 'sandwich' Enzyme-linked Immunosorbent Assay (ELISAs).

INFLAMMATORY RESPONSE

The properties of abutment may play an important role in crestal bone stability and healthy soft tissues which are considered for the long-term success of implant-supported restorations.(7) Peri-implantitis is defined as an inflammatory reaction around osseointegrated implant in function with loss of supporting bone. If inflammation is located only the soft tissues surrounding implants, it is described as peri-implant mucositis.(2) Two main etiological factors that contribute to the inflammation in peri-implant tissues are bacterial infection and biomaterial type of

abutments used.(3) Plaque accumulation can cause the inflammation of subepithelial connective tissue with inflammatory cell infiltrations. Following this, the connective tissue seal is loosely fixed. The clinical and radiographical signs of tissue destruction can be observed. (37) In recent *in vitro* and *in vivo* studies found that the surface roughness and surface texture in the micrometer may impact on the early healing by influencing attachment, orientation, proliferation and metabolism of epithelial and connective tissue cells.(3) Increased surface roughness has also been associated with increased osseointegration of dental implant. On the contrary, a higher surface roughness increases the biofilm formations especially transmucosal abutment surface.(40) Bollen et al. in 1997 determined the threshold surface roughness value of bacterial retention on titanium. The threshold value was R_a = 0.2 μm. Decreasing in surface roughness below this threshold, no or only minor influence of the surface topography occurred on plaque accumulation.(41) In the same way, the effect of surface roughness on early plaque retention on titanium conducted by Rimondini and colleagues concluded that titanium surface with $R_a \leq 0.088$ μm and $R_z \leq 1.027$ μm prevented plaque accumulation and maturation at 24-hour time period.(42) Therefore, not

only the biocompatible materials but also the surface of prostheses component should be considered in order to obtain healthy soft tissue seal.

The elevated levels of inflammatory biomarkers in peri-implant crevicular fluid is correlated with the destructive processes of peri-implant soft tissues. In clinically healthy periodontal tissues, inflammatory cytokines are present in low quantities being as factors mediating normal tissue homeostasis. Among the numerous biomarkers, proinflammatory cytokine [e.g. tumor necrosis factor (TNF)-α, interferon-γ, interleukin IL-1β, IL-6, IL-12, IL-17 and RANKL], anti-inflammatory cytokines (e.g. IL-4, IL-10 and IL-1 receptor antagonist) and chemokines [e.g. IL-8, monocyte chemoattractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α] have been suggested to be important mediators of inflammation and immunity in the pathogenesis of peri-implantitis. Several researchers have attempted to use the biological markers to define the health status of dental implants.

Interleukin-1 (IL-1) is produced mainly by macrophages and various kinds of cells such as neutrophils and fibroblasts. There are two IL-1 ligands with agonist activity, IL-1α and IL-1β.(43) IL-1α play an important role during wound healing enhancing wound epithelialization.

IL-1β is the major inflammatory cytokine occurring in the periodontitis. It regulates a biological effect including stimulation of collagenase and prostaglandin E2 (PGE2) synthesis, osteoclastic bone resorption and tissue destruction. The study by Kao et al showed the increased level of IL-1β at peri-implantitis sites. They concluded that the result of higher IL-1β levels in failing implants can distinguish healthy versus diseased implants.(44) Similarly, there were significant differences in IL-1β levels in PICF from peri-implantitis sites in Masashi et al study that compared peri-implantitis, mucositis and healthy implants after loading 35.8 months in average.(45) Ataoglu et al. study exhibited the IL-1β and TNF-α levels in inflamed gingival tissue had higher than those of in non-inflamed or slightly inflamed peri-implant tissue. This finding indicated that neutrophil elastase activity and IL-1β levels in PICF may be used to evaluate the status of implant health.(46) Regarding the comparison of proinflammatory cytokine levels in dental abutment, the results showed that IL-1β and IL-6 levels in ceramic abutment were significantly lower than titanium abutment. Moreover, the higher levels of IL-6 in titanium and ceramic abutment compared with IL-1β levels.(47)

Interleukin-6 (IL-6) is a proinflammatory cytokine synthesized by monocytes, endothelial cells and fibroblasts, which stimulates B and T cells activation in both acute and adaptive immune system.(48, 49) The study of Yuanyuan et al. concluded that IL-6 levels in peri-implantitis and peri-mucositis higher than healthy implants. There were significant difference in IL-6 levels that associated with the plaque index, gingival index, probing depth and bone loss.(48) Furthermore, a significant difference was found in the level of IL-6 around peri-implantitis and healthy implants as well as between peri-implantitis and healthy teeth.(50)

Interleukin-8 (IL-8) is secreted by macrophages and epithelial cells. The expression of IL-8 triggered by IL-1 and TNF-α. The primary action is a chemotaxis for polymorphonuclear leukocytes. Getulio et al. have analyzed the comparison of cytokine in PICF and GCF in healthy patients. Their results showed the level of IL-8 and IL-α were higher in GCF than PICF at one, two, six and twelve months. In contrast, the levels of IL-6, TNF-α, INF-γ were not significantly different and did not changed over time between GFC and PICF.(51) Norbert et al. concluded the correlation in the expression of five biomarkers (Il-1RA, Il-8, G-CSF, MIP-1β, and TNF-α) at zirconia implants and teeth, four biomarkers (L-1RA, IL-8, GM-CSF, and MIP-1β) at zirconia

and titanium implants as well. Zirconia implants had the levels of IL-1β and TNF-α more than at teeth. However, no significant differences were found between zirconia and titanium implants.(52) Lists of cytokines and chemokines in this study were concluded in the table 2.

Table 2 List of inflammatory mediators (38, 43, 53, 54)

Group	mediators	Principal Cell Sources	Target / Action and Biologic effects
IL-1 family	IL-1β	monocyte, macrophage, epithelial cells, fibroblasts and dendritic cells	increase inflammatory cell migration, increase osteoclastogenesis, neutrophil production, induce the secretion of IL-8
Chemokines	IL-8	epithelial cells and macrophage	attracts PMN to the inflammation site, angiogenic activity, increases osteoclast differentiation and activity
T_{h2}	IL- 6	T and B cells, macrophages and epithelial cells	stimulate B cell differentiation and T cell activation, induce acute phase response

RESEARCH QUESTIONS

Do the peri-implant soft tissue reactions and cytokine expressions around different abutment materials from peri-implant crevicular fluid collection demonstrate similar characteristics?

RESEARCH OBJECTIVES

The aim of this study is to evaluate the effect of 3 different types of abutment materials, which are titanium, zirconium oxide and gold alloy on the cytokine expressions in the peri-implant soft tissue by using peri-implant crevicular fluid collection.

HYPOTHESIS

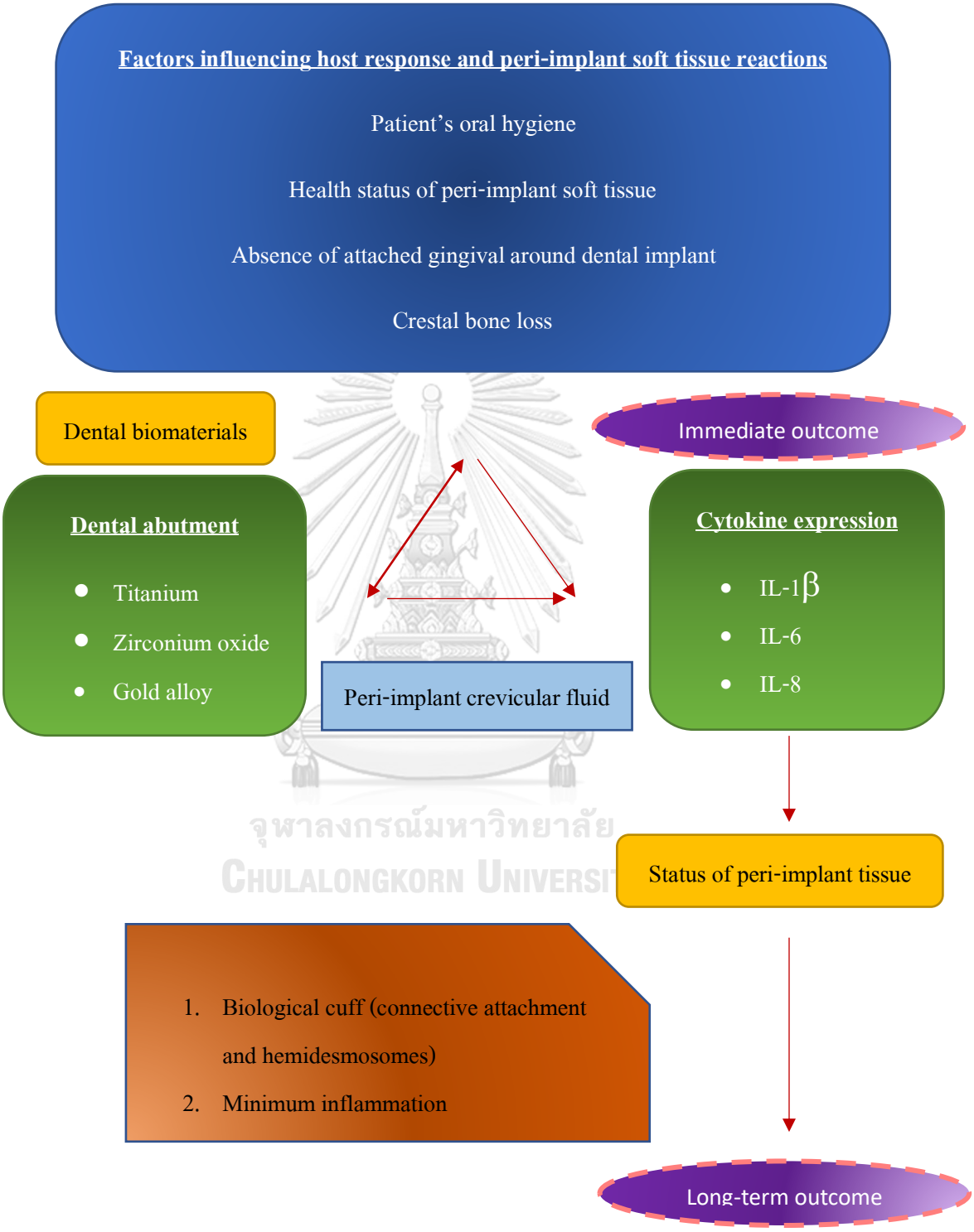
Null hypothesis

The peri-implant soft tissue reactions and cytokine expressions around different abutment materials demonstrate similar characteristics.

Alternative hypothesis

The peri-implant soft tissue reactions and cytokine expressions around different abutment materials demonstrate different characteristic at least one group.

CONCEPTUAL FRAMEWORK



KEY WORDS

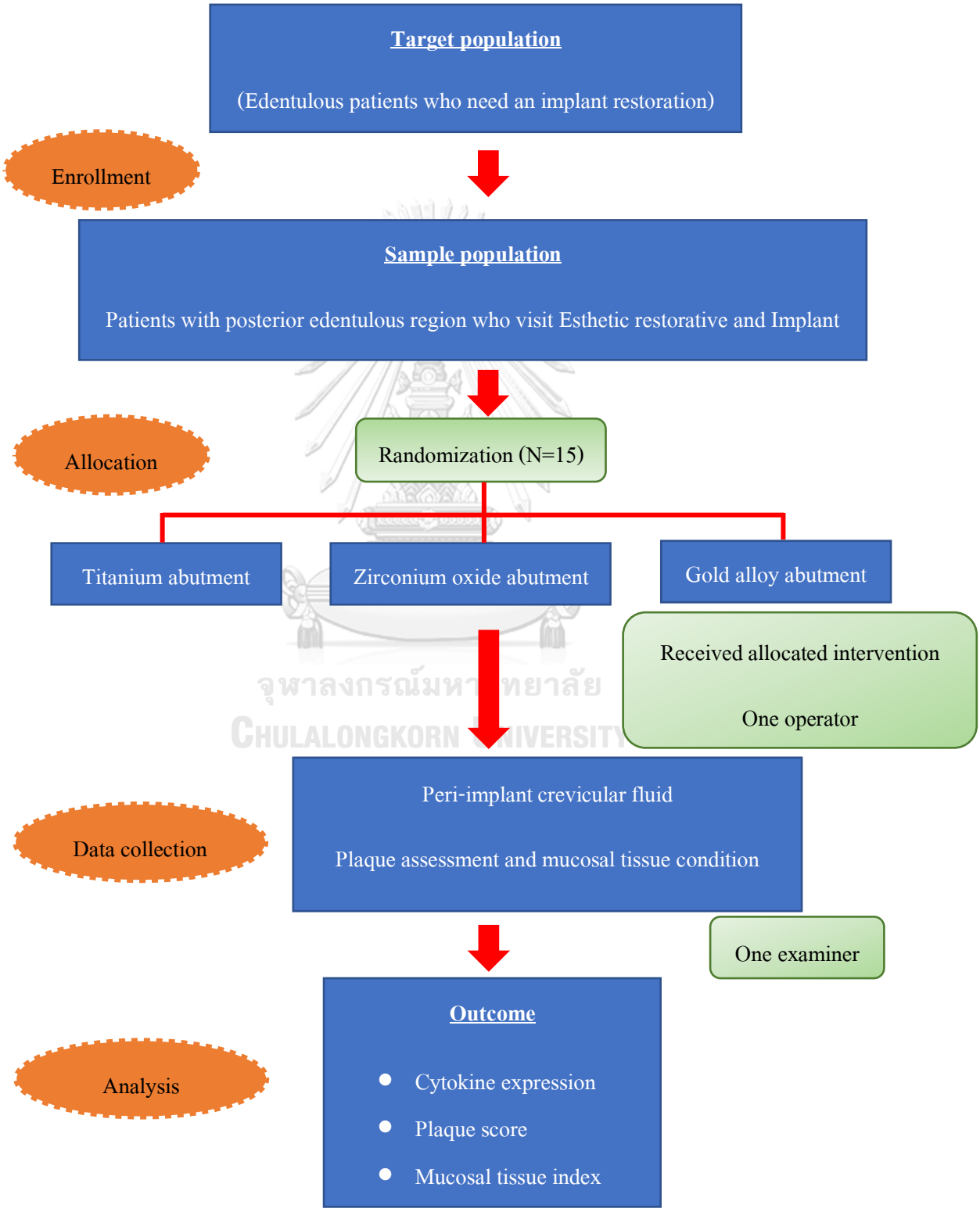
Dental implant abutment, Cytokine level, Peri-implant crevicular fluid, Enzyme-linked immunosorbent assay (ELISA)

RESEARCH DESIGN

The study was carried out as a single-blind randomized controlled trial. The aim of this study was to compare the clinical evaluation among the intervention (zirconium oxide and gold alloy) and the standard abutment material (titanium) in terms of cytokine expression. The patients were blinded to the type of abutments used.

RESEARCH METHODOLOGY

DIAGRAM OF STUDY DESIGN



POPULATION AND SAMPLE

Target population:

Edentulous patients who need an implant restoration.

Sample population:

The patients with posterior edentulous region who visit Esthetic restorative and Implant dentistry clinic, Chulalongkorn University and met the following criteria. Patients who were planned to receive placement of AstraTech OsseoSpeed™ EV 4.8 dental implant were examined. All participants were explained about the study if they fulfill the inclusion criteria. Participants were asked to sign informed consent before enrollment in the project.

Inclusion criteria

- Healthy patients over 21 years of age
- Having at least 1 implant-supported fixed partial prosthesis
- Implant fixture placed at least 3 mm deeper from soft tissue margin
- Sufficient residual bone volume for implant with diameter of 4.8 mm

- Inter-arch space > 5mm

- Not underwent previous periodontal treatment for at least 3 months

Exclusion criteria

- Patient presented with systemic disease

- Having immunosuppressant medications or antibiotic within 3 months

- Pregnancy and lactating

- Smoker

- No conditions requiring chronic routine prophylactic use of antibiotics

- Being a handicap that would interfere with the ability to perform adequate oral hygiene and attending all follow-up procedures

SURGICAL PROCEDURES AND ALLOCATION TECHNIQUE

The patients who met the above selection criteria and gave informed consent for participation were recruited into the study. The surgical preparations of implant placement were performed by postgraduate students who studied in the Esthetic Restorative and Implant Dentistry program under the supervision of an experienced surgeon. Each patient was randomized into either the treatment or the control group. Dental abutment was immediately installed instead of healing abutment and was reduced the height to avoid the contact with the opposing teeth both centric and eccentric movement. (Fig 2) All subjects were prescribed antibiotic (Amoxicillin 500 mg tid) for 7 days and advised to rinse 0.2% chlorhexidine mouth wash for 2 weeks. The oral hygiene instructions were informed. Tooth brushing with modified bass technique and interdental cleaning with floss were recommended twice a day. A clean gauze pad wrapped around finger was advised to wipe in particular area of implant abutment.

The assignment of the abutment to a group was determined by the process of simple randomization with picking up an envelope so that each tooth had an equal chance of being assigned to either the intervention or the control group. The peri-implant crevicular fluid

collection was performed by a single operator.

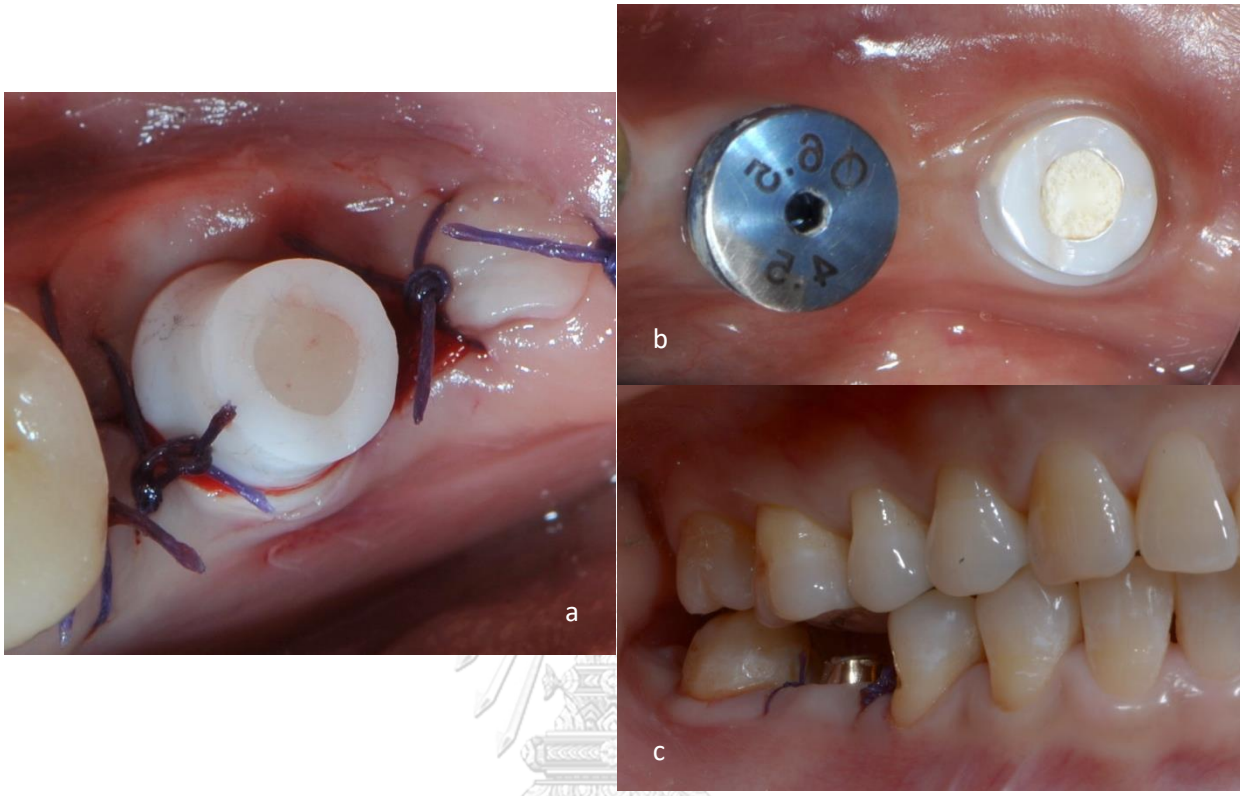


Figure 2 Day-0 Surgical Implant placement

a. *Abutment was installed immediately after implant placement.*
b. *Randomized abutment was inserted instead of healing abutment.*
c. *The height of abutment was reduced to avoid the contact to opposing teeth*

INTERVENTION

Control Group: Titanium abutment

Treatment Group: zirconium oxide and gold Alloy abutment

Table 3 Type of implant abutments

Group 1	Titanium The TiDesign™ EV 4.8 triangular shaped abutment with a diameter of 5.5 mm (product code 25340) for OsseoSpeed™ EV 4.8 implant from DENTSPLY Implant	
Group 2	Zirconia The ZirDesign™ EV 4.8 triangular shaped abutment with a diameter of 5.5 mm (product code 25322) for OsseoSpeed™ EV 4.8 implant from DENTSPLY Implant	

Group 3	Gold-alloy The CastDesign™ EV 4.8 abutment with a diameter of 5.1 mm (product code 25328) for OsseoSpeed™ EV 4.8 implant from DENTSPLY Implant will be used and casted with gold type4 to the same shape and diameter as the abutment in group1 and group2.	

In order to control the surface roughness of implant abutment, all experimental abutments were measured the roughness value which were a profile roughness (R_a) and surface roughness (S_a) before allocating to the patients. Since gold alloy abutment was casted from dental laboratory, but titanium and zirconium oxide abutment were manufactured from the company. To reduce the effect of surface roughness on biofilm formation which might be involved the increase of cytokine levels, each implant abutment had to be polished until no significant difference in

surface roughness. Contact profilometer machine (Talyscan 150, Taylor Hobson, England) was selected to scan the surface of implant abutment, setting length X = 1 mm Y = 1 mm, spacing X = 0.5 μm Y = 5 μm and speed 1000 μm/sec. The raw data and statistical analysis of surface roughness in each abutment reported in table 10.

SAMPLE SIZE CALCULATION

Power analysis was performed to determine sample size in order to detect a true effect of this study. This analysis normally was used to design a study prior to data collection process started. Since there were no previous studies within the same topics. We used data from pilot study where samples were collected at week 4 after an individual implant abutment was installed. It helps us to determine whether we should recruit more participants in order to yield a real effect from the population. The data in this analysis consisted of total 12 participants with 4 participants from each abutment material group. With actual data points and standard deviation of the pilot study data, F family of tests of Apriori one-way ANOVA, one of power analysis types, results were showed in the chart (Fig 3-5). We also set alpha value = 0.05 as a parameter in G-Power application. With this alpha value, it means we are able to accept that there might be 5% probability of inaccurately reject null hypothesis. We simply see from the test results that sample size of 12 provided power value 0.95 which was very high from IL-1β. For IL-6 and IL-8 measurements, there were some data points that were unable to detect from ELISA. The IL-6 and IL-8 values in previous literatures were therefore took to estimate the best suitable values for

where the values were missing in order to perform power analysis on both measures. With actual and estimated IL-6 and IL-8 values, power analysis suggested that we need at least 15 participants in order to obtain statistical power of 0.86 and 0.71 for IL-6 and IL-8, respectively. We actually reached 15 participants at the end of this study. Although we aimed to earn at least 0.80 statistical power for all three measures, we were unable to find additional participants in order to meet minimum number of samples for IL-8 due to scarcity on enrollment human participants. With IL-1β and IL-6 alone, the study with total of 15 participants has enough statistical power which are 0.986 and 0.86 for IL-1β and IL-6, respectively (Fig 3,4).

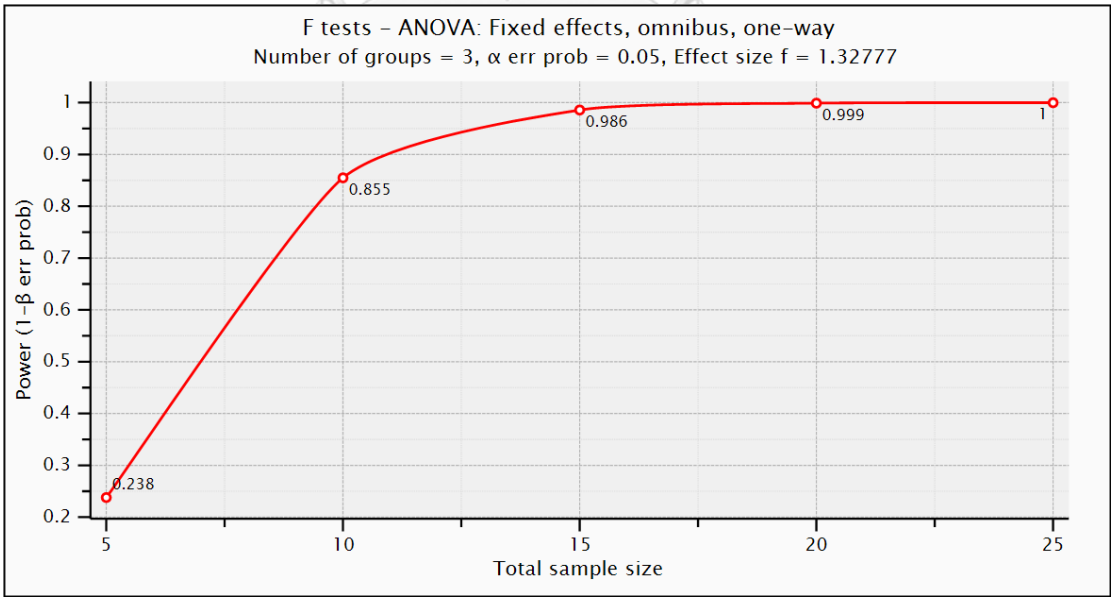


Figure 3 Apriori one-way ANOVA for power analysis of IL-1β

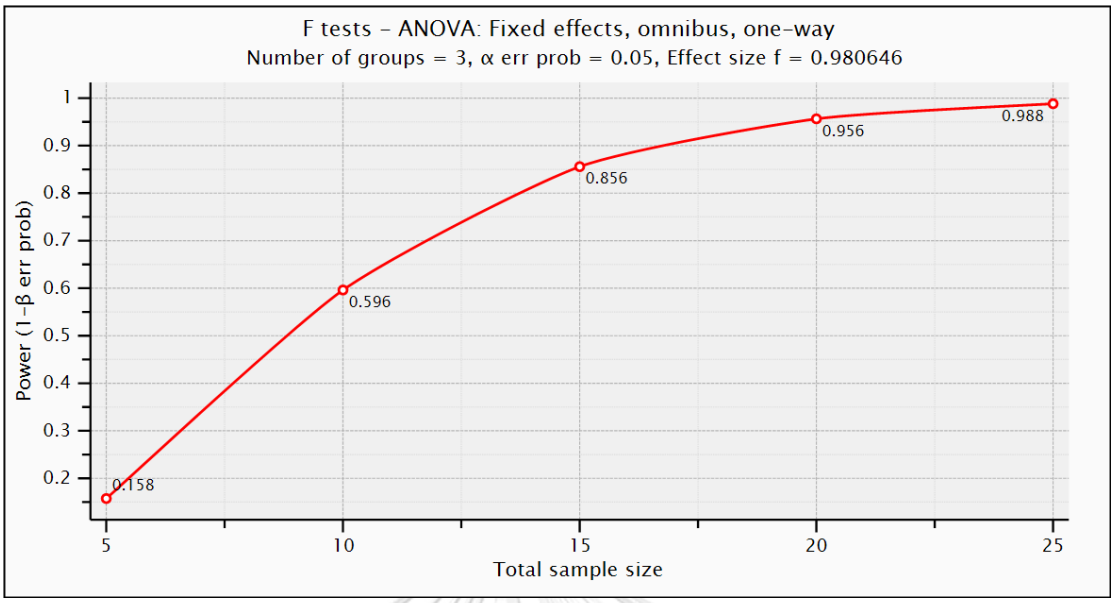


Figure 4 Apriori one-way ANOVA for power analysis of IL-6

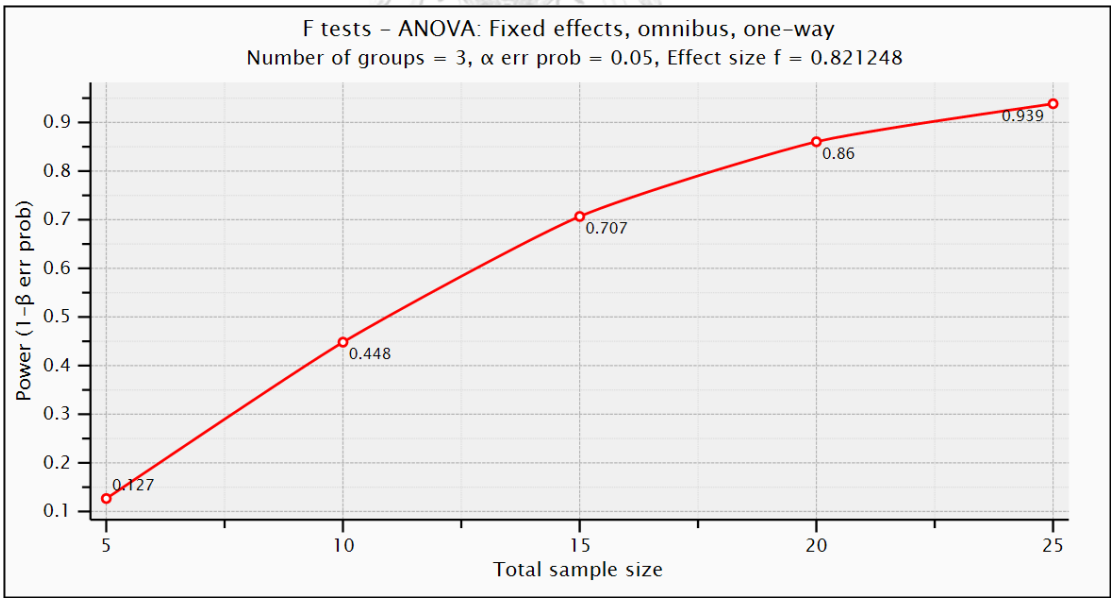


Figure 5 Apriori one-way ANOVA for power analysis of IL-8

DATA COLLECTION

EVALUATION OF THE ORAL HYGIENE

Plaque Assessment around dental implant (55)

Following abutment connection, a plaque control program was initiated and maintained for 10 weeks. At each visit during the observation period the oral hygiene level was evaluated according to a 3-point scale.

Table 4 Plaque score

Score	Description
0	No visible plaque
1	Local plaque accumulation
2	General plaque accumulation greater than 25%

EVALUATION OF THE PERI-IMPLANT MARGINAL TISSUES

Mucosal Conditions around dental implants (56)

A simplified gingival index that has been proposed by Apse and associated, was used to assess mucosal tissue condition around dental implant.

Table 5 Mucosal tissue condition index

Score	Description
0	Normal mucosa
1	Minimal inflammation with color change and minor edema
2	Moderate inflammation with redness and edema
3	Severe inflammation with redness, edema, ulceration and spontaneous bleeding without probing

PERI-IMPLANT CREVICULAR FLUID (PICF) COLLECTION

Peri-implant crevicular fluid was collected at week 4, 6, 8 and 10 after the implant placement and obtained from four sites (mesial, distal, buccal and lingual). Supragingival plaque or calculus was carefully removed. The implant was isolated with cotton wool rolls and air dried. The paper points size M (Kerr, CA, USA) were introduced into the sulcus/pocket in the apical direction, until a little resistance was felt and kept in the site for 30 seconds. Strips visibly contaminated with saliva and/or blood was discarded. PICF absorbed from each strip was stored in 1.5 mL plastic tube containing 1000 µl of phosphate buffer saline (PBS), pH 7.2, supplemented with protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). The samples were frozen at -80°C for later analysis (Fig 6).

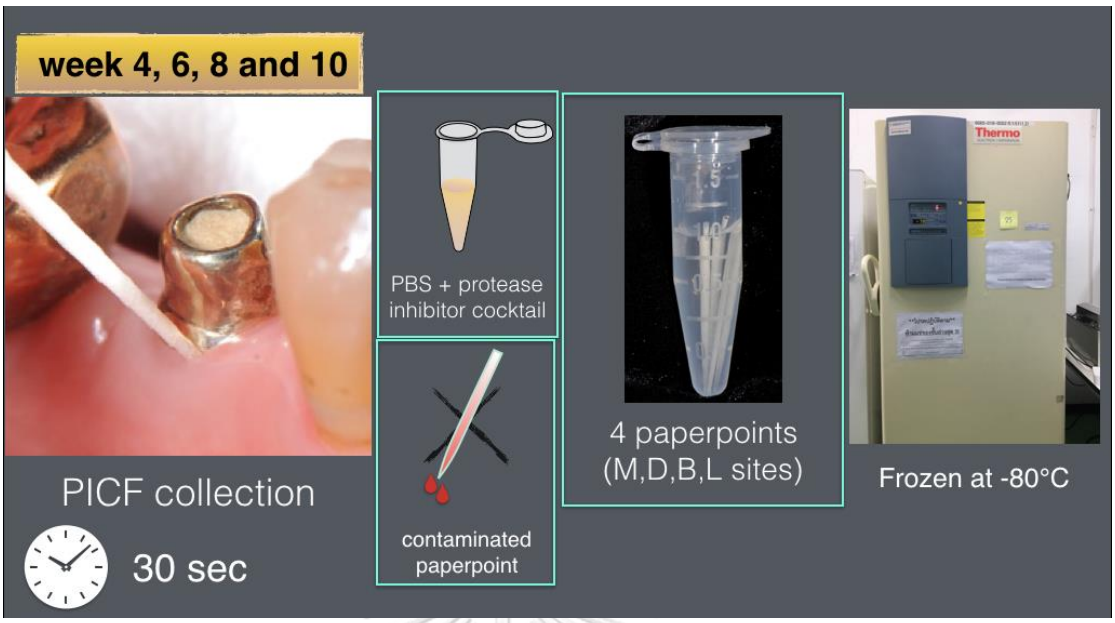


Figure 6 Peri-implant crevicular fluid collection

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Proinflammatory cytokine (IL-1β, IL-6) and chemokine (IL-8) concentrations in PICF were assessed using com`(57)mercially available ELISA kits. (ELISA MAX™ Deluxe set human; Biolegend, USA) The kits use a quantitative "sandwich" enzyme immunoassay technique. This kit contains assay human capture antibody, human detection antibody, human standard, streptavidin-HRP, reagent diluent, substrate solution and 96 well microplates. The assessment was performed according to the manufacturer's instructions. Firstly, 96 well microplates were prepared by coating of the diluted capture antibody. Then, the sealed plates were incubated

overnight at room temperature. After that each well plate was aspirated and rinsed with wash buffer. Secondly, the assay diluent A was added to each well and incubated at room temperature for one hour with shaking on a plate shaker. The plates were now ready for sample additions and incubation for 2 hours with shaking. Then the detection antibody was added and incubated 1 hour at room temperature. The working dilution of Streptavidin-HRP was added and incubated 30 minutes with shaking. Finally, the substrate solution and stop solution were used. The well plates were determined the optical density using spectrophotometry 450 nm. Concentrations in each sample were determined by generation of standard curve. Then, the total amount of IL-1β, IL-6 and IL-8 in each sample was defined as pictograms per milliliter (Fig 7).

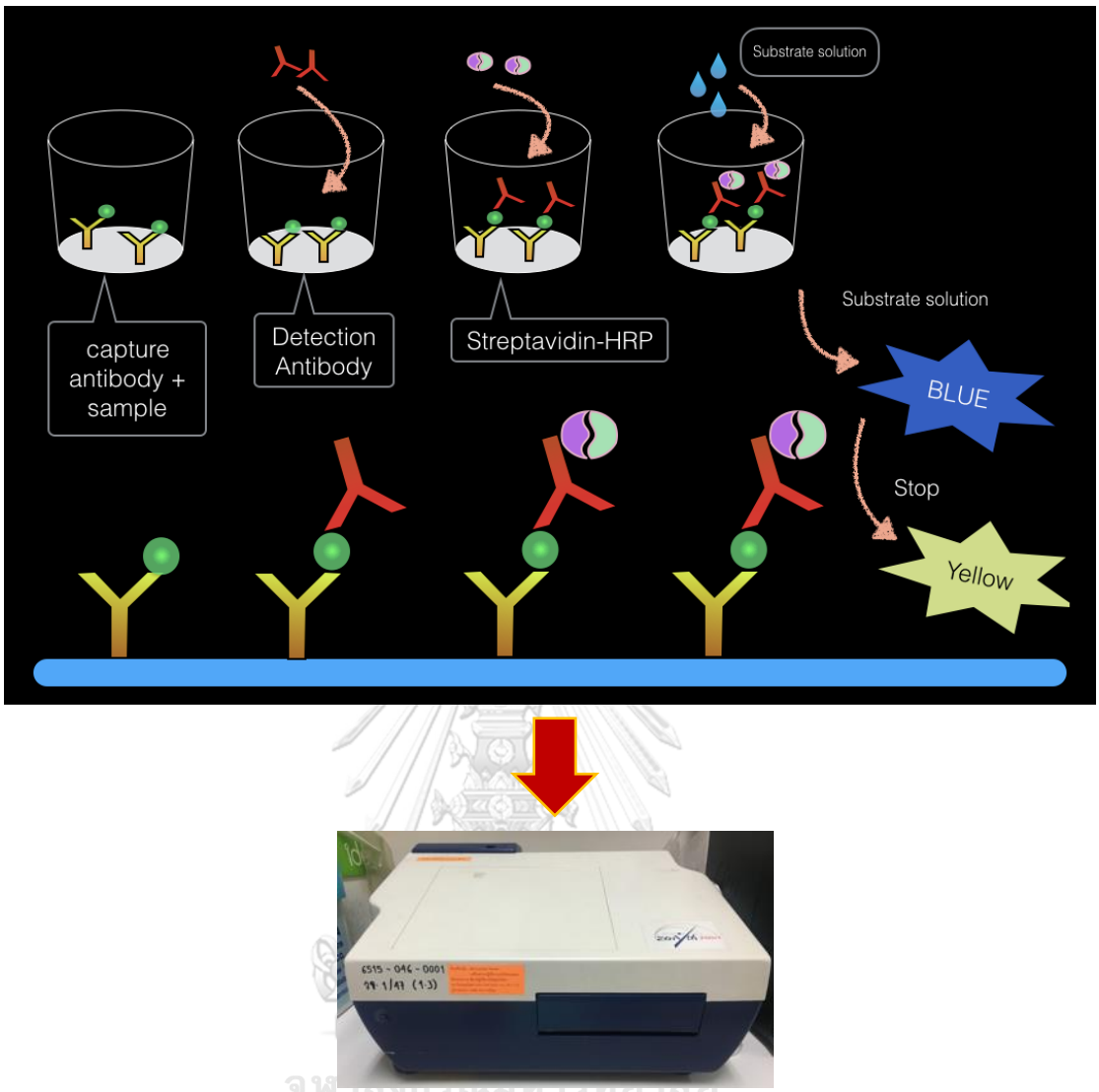


Figure 7 Enzyme-linked immunosorbent assay (ELISA)

DATA ANALYSIS

Table 6 showed the demographic variables in this present study with statistics. The outcome variables demonstrated in table 7.

DEMOGRAPHIC VARIABLES

Table 6 Demorgraphic variables

Variable	Type of Variable	Statistics
Sex	Categorical: dichotomous	Mode
Age	Continuous	Mean, S.D.
Edentulous region (Maxilla, Mandible)	Categorical: dichotomous	Mode

OUTCOME VARIABLES

Table 7 Outcome variables

Variable	Type of Variable	Statistics
IL-1β IL-6 IL-8	Ratio Scale	• Kruskal-Wallis one-way analysis of variance and pair-wise with Dunn test
Plaque score	Categorical	• Mode, Percentage
Mucosal tissue index	Categorical	• Mode, Percentage

RESULT

1. Demographic data of study sample

15 Astra Tech OssepSpeed™ EV implants with diameter of 4.8 mm were placed in the first and second molar regions. The number of implant abutments in each group was divided equally by randomization technique. Table 8 described the demographic data of the study sample.

Table 8 Demographic data of study sample

Parameter	**Subjects**	
Total population	15	
Age, mean ±SD	Titanium	57.4±3.78
	Zirconium oxide	63.2±8.23
	Gold alloy	48.2± 14.38
Gender	Male 9, Female 6	
Edentulous region	Lower left 4, Lower right 6, Upper left 3, Upper right 2	

2. Clinical parameters

Clinical parameters and cytokine expression in peri-implant crevicular fluid (PICF) were investigated at healing period of 4, 6, 8 and 10 weeks. Plaque index and mucosal condition score were depicted in Figure 8 and 9. Regarding to plaque assessment, score of 2 was found only in gold alloy abutment (20%) at week 4. Then plaque scores were equally 20% for score 0 and 80% for score 1 at weeks 6,8 and 10. While plaque score of zirconium abutment performed better than other materials every week. None of the groups demonstrated moderate to severe inflammation of mucosal tissue. However, gold alloy abutment received higher percentage of mucosal tissue condition score of 1 than titanium and zirconium oxide.

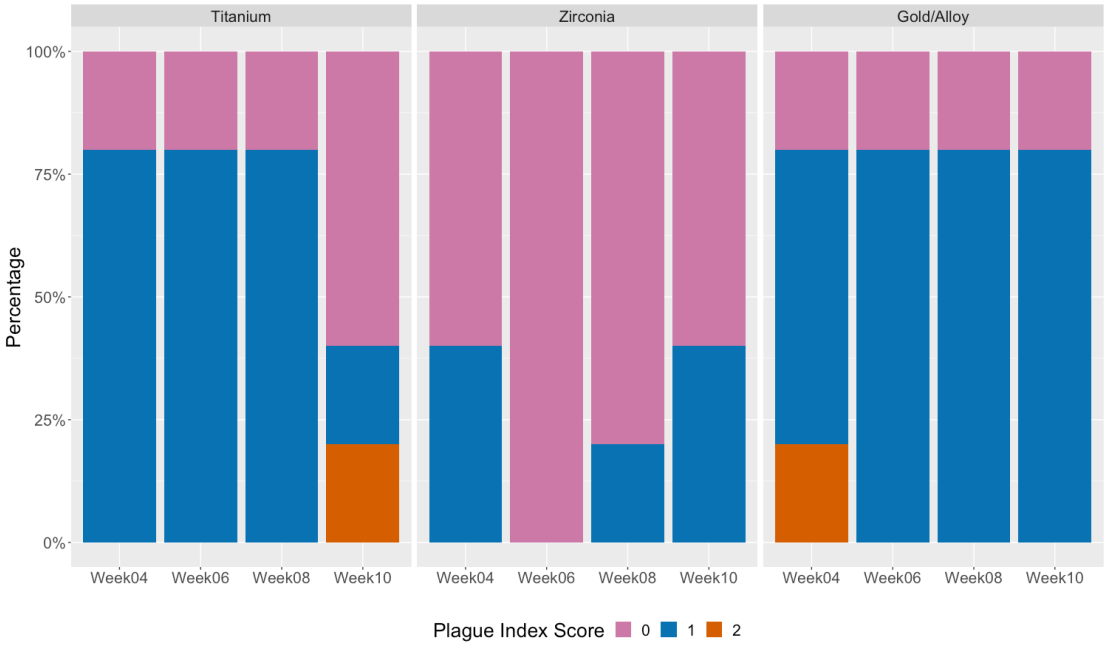


Figure 8 Descriptive results of the plague index score

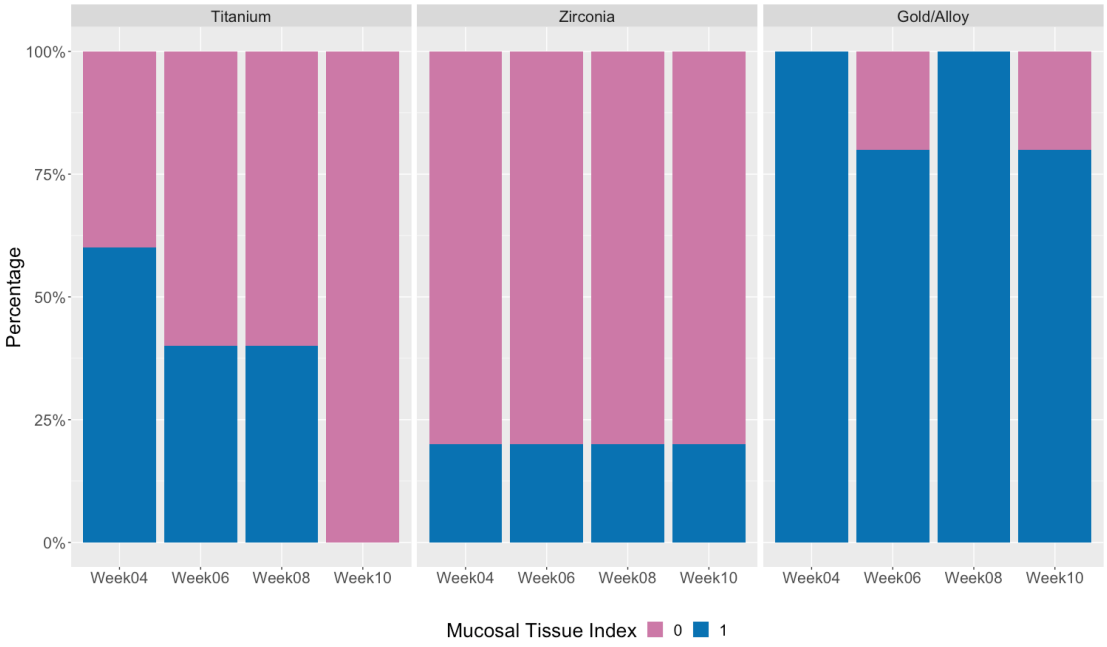


Figure 9 Descriptive results of the mucosal tissue index

3. Concentration of IL-1β, IL-6 and IL-8

The present study compared the concentration of pro-inflammatory cytokines and chemokines in different types of abutment material and time points. This study had small sample sizes in each group. Hence, Kruskal-Wallis non-parametric test was used to assess the statistical analysis. Dunn test was also used in pairwise comparisons. The concentration of IL-1β, IL-6 and IL-8 expressed in pg/ml are presented in Figure 10, 12 and 13.

Following healing times, mean IL-1β values in titanium group were 65.59±58.61, 52.63±59.11, 69.97±78.57 and 224.16±98.26 pg/ml, respectively. For zirconium oxide abutment, mean values of IL-1β were 63.63±20.87, 54.31±23.18, 127.86±51.74 and 226.25±89.25 pg/ml at week 4, 6, 8 and 10, respectively. While the mean concentration of IL-1β in gold alloy group were 226.68±63.39, 202.04±85.02, 196.95±55.05 and 261.90±43.08 pg/ml. At weeks 4, 6 and 8, IL-1β levels were significantly higher in gold alloy abutment than in titanium and zirconium oxide abutment (p-value < 0.05). However, week 10 values of IL-1 levels were similar (p-value > 0.05) (Fig 10).

The comparison of IL-1β between types of abutment revealed gold alloy were significantly higher than titanium and zirconium oxide groups at week 4 and 6. Whereas week 8 showed gold alloy had higher IL-1β than titanium only (p-value < 0.05) (Fig 11).

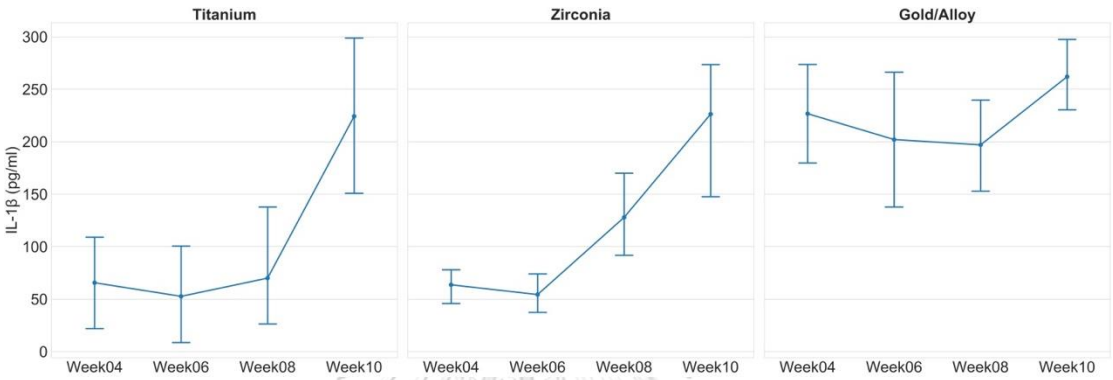


Figure 10 Time-dependent level of IL-1β (pg/ml) in different implant abutments. Values are presented as mean±SD.

IL-1β levels were significantly higher in gold alloy abutment than in titanium and zirconium oxide abutment at weeks 4,6 and 8 ($p<0.05$). While no significant changes were observed at week 10($p>0.05$).

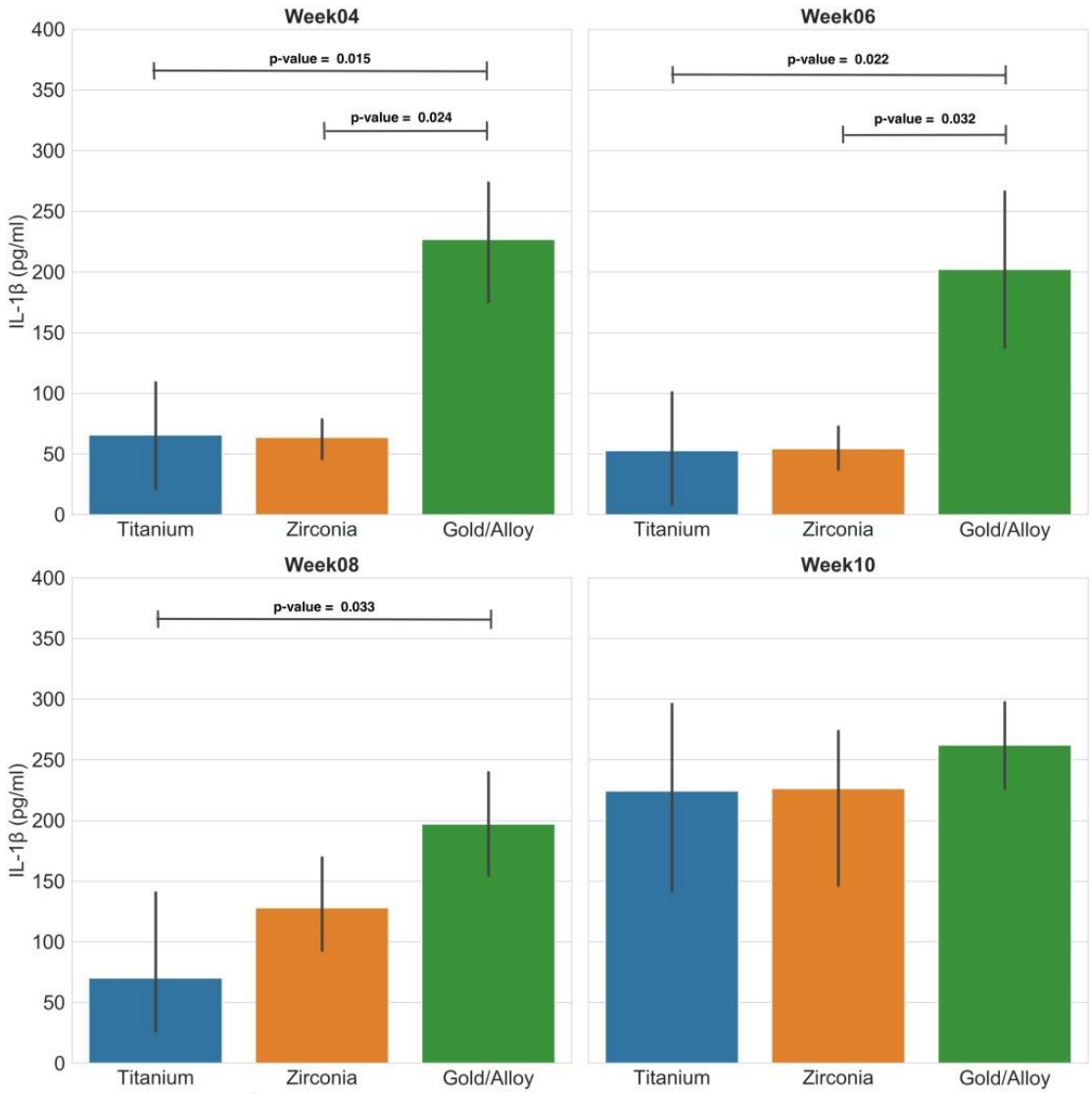


Figure 11 Comparison of the concentration of IL-1β (pg/ml) between abutment materials at weeks 4,6,8 and10. Values are presented as mean±SD.

*Statistical significances were observed in pairwise using Dunn test (*p<0.05.)*

With respect to IL-6, the concentration of IL-6 was more prominent around gold alloy abutment when compared to titanium and zirconium oxide abutment which could not detected in peri-implant crevicular fluid at weeks 4, 6 and 8 (Fig 12).

The mean concentration of IL-8 (pg/ml) according to time points were 224.32±118.07, 112.5±3.58, 90.73±42.05 and 139.25±97.53 in titanium abutment, 366.9±198.81, 239.87±334.98, 84.65±44.86 and 152.52±79.31in zirconium oxide group whereas gold alloy abutment showed 349.32±193.23, 126.59±132.19, 98.73±88.41 and 57.04±44.92, repectively. In general, the levels of IL-8 were decreased from week 4 to week 6 and quite stable until week 10. However the difference was not statistically significant in the levels of IL-8 between groups of abutment (Fig 13).

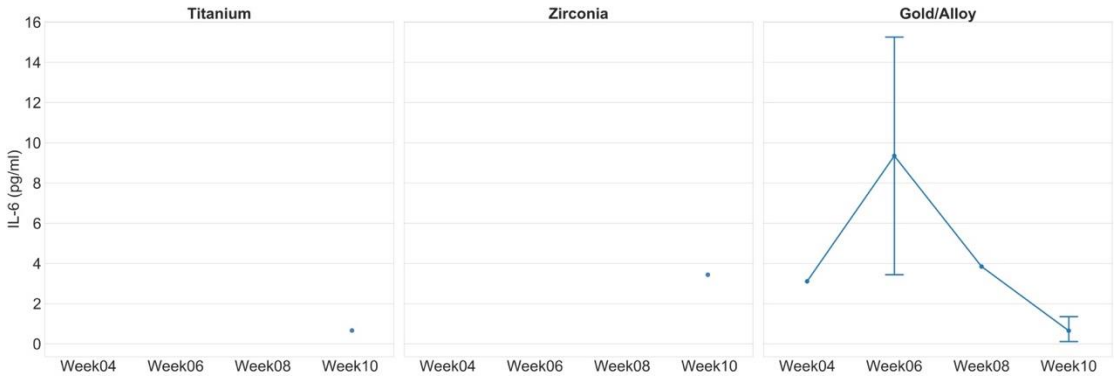


Figure 12 Time-dependent level of IL-6 (pg/ml) in different implant abutments. Values are presented as mean±SD.

Gold alloy abutment had pronounced expressions of IL-6 when compared to titanium and zirconium oxide abutment which could not detected in PICF at weeks 4,6 and 8.

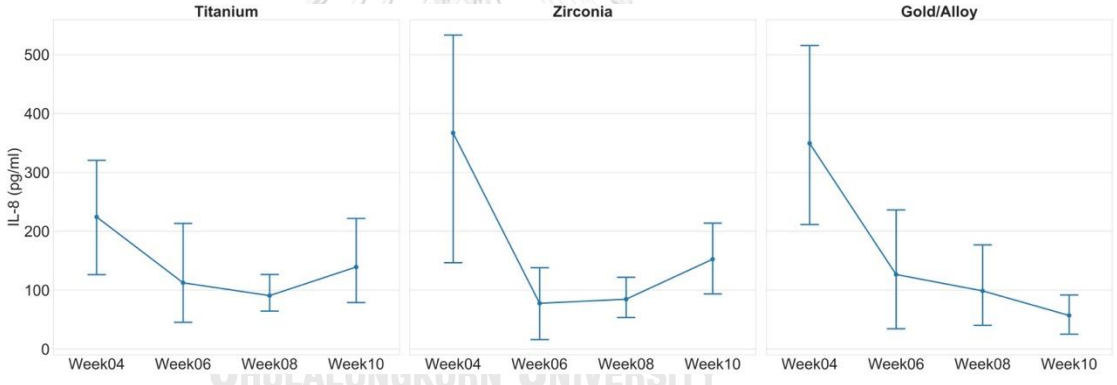


Figure 13 Time-dependent level of IL-8 (pg/ml) in different implant abutments. Values are presented as mean±SD.

The analyses compared values of weeks 4,6,8 and 10 showed there were no significant differences in IL-8 between abutment materials ($p>0.05$).

4. Percent of control

Percent of control presented in table 9 and figure 14 using formula :

$$\text{Percent of change} = \frac{\text{Present Value} - \text{Pilot Study Value}}{\text{Pilot Study Value}} \times 100\%$$

Table 9 showed the individual' values in percent of change following healing time. Graphs in figure 14 displayed the trend of percent of control of individual samples in 3 types of abutment materials.

Table 9 Percent of change from week 4 in the individual samples

Material	Patient No.	Week 4	Week 6	Week 8	Week10
Titanium	6	0	-99.06	-67.52	103.3
Titanium	8	0	-89.81	154.3	309.94
Titanium	12	0	489.62	55.67	1577.66
Titanium	14	0	6.93	-31.47	967.84
Titanium	21	0	5	57.88	34.47
Zirconium oxide	1	0	-70.08	30.83	238.87
Zirconium oxide	5	0	59.97	198.85	668.85

Zirconium oxide	7	0	-37.93	95.94	-6.34
Zirconium oxide	11	0	70.93	309.43	398.03
Zirconium oxide	18	0	-22.35	1.16	247.01
Gold alloy	4	0	-7.82	-39.83	-25.75
Gold alloy	10	0	-48.34	35.54	44.19
Gold alloy	13	0	34.5	-47.27	36.12
Gold alloy	17	0	11.91	66.93	81.59
Gold alloy	24	0	-35.47	-29.98	-11.57

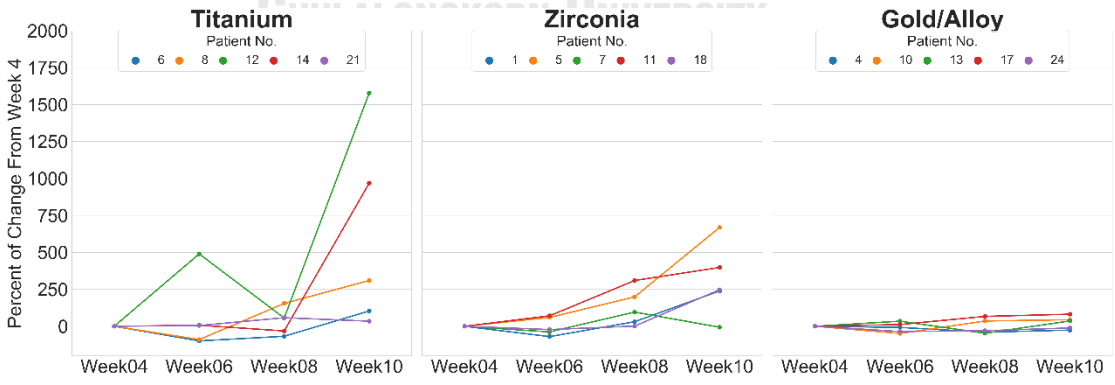


Figure 14 Percent Change (Baseline = week 4)

DISCUSSION

Several periodontal indices have been suggested as diagnostic tool for assessing soft and hard tissue condition. Common clinical indicators such as bleeding on probing and probing depth are not reliable tools for the status of the peri-implant tissue. Healthy peri-implant mucosa can present an increase of probing depth over $\geq$ 4 mm and is not associated with bone loss. Correspondingly, bleeding on probing may reflect the nature of the scar tissue of peri-implant mucosa.(2, 58) The analysis of peri-implant crevicular fluid (PICF) has been used to assess the inflammatory mediators. It is obvious that peri-implant crevicular fluid (PICF) provides the essential information regarding the status of peri-implant tissue. Collection of peri-implant crevicular fluid is a noninvasive and relatively simple method to measure the immune function. This procedure can be used as an early detector of periodontal disease and healing or periodontal tissues.(39) There is increase in the production of various cytokines and adhesion molecules that promote the extravasation of leukocyte to the inflammatory site. Implants with peri-implantitis are associated with higher levels of proinflammatory cytokines than healthy implants.(13) The clinical conditions and soft tissue status based on the peri-implant crevicular fluid (PICF) may

predict and monitor the peri-implant tissue response. In the present study, the cytokine expression and the peri-implant mucosa reaction including plaque assessment and mucosal condition were evaluated. This experimental study used a sandwich ELISA method with a single array in each cytokine to assess the cytokine levels. Unlike, indirect ELISA, antigens with low or unknown concentration in the sample can be detected because the capture antibody only grabs the interested antigen and other proteins in the sample are washed away. This technique also has an individual standard curve. Sandwich ELISA is suited to the analysis of complex samples such as the measurement of cytokine levels in the immune response.

All subjects in this study had similar implant system (AstraTech OsseoSpeed^{TM} EV) with diameter 4.8 mm and implant-abutment connection. Each implant abutment which was immediately installed instead of healing abutment was reduced the height to avoid the contact with the opposing teeth in all directions. Additionally, patients with healthy status, non-smoking and sufficient bone with no grafting were included in this current study. These complements may induce proinflammatory cytokine and chemokine, thus serving as the control factors. (58)

To examine the host immune response around different types of implant abutment, the proinflammatory cytokines and chemokine in peri-implant crevicular fluid (PICF) were assessed in this study. Interestingly, the overall trend of IL-1β throughout the follow up period gradually decreased from the initial healing period (week 4) to week 6 and then increased accordingly from week 6 until week 10. We can infer that surgical trauma stimulated the release of proinflammatory cytokine during initial phase of peri-implant wound healing, followed by bone remodeling activity and peri-implant soft tissue maturation around transmucosal area.(57, 58) Based on data from previous study, IL-1β levels in healthy implants, peri-implant mucositis and peri-implantitis demonstrated briefly in range 10-120 pg/ml, 100-325 pg/ml and 250-900 pg/ml, respectively.(44, 59, 60) Even though the expression of IL-1β in peri-implant crevicular fluid represented highest values in all materials at week 10, almost their levels reported not exceeding to peri-implantitis. Likewise, the expressions of IL-6 and IL-8 in different materials and time points were observed in healthy to peri-implant mucositis level.

IL-6 levels in titanium and zirconium oxide abutment were not detected during 4-8 weeks of healing but some sample displayed at week 10. On the contrary, the concentration of IL-6 in gold

alloy exhibited in all weeks with a peak expression at week 6. IL-6 have been observed in response to acute surgical trauma in both soft and hard tissue. It stimulates B cell differentiation and T cell activation as well as links innate to adaptive immune systems by changing the nature of leukocyte infiltration from PMNs to macrophages.(58, 61)

Previous studies claimed that there are the relationships between immune cells, inflammatory mediators and bacteria.(62) Plaque accumulation is correlated with the inflammation of soft tissue. In our study, higher level of IL-1β and IL-6 during week 4-8 concurred with higher plaque assessment score and mucosal tissue index found in gold alloy abutment group, while the result showed no significant difference in IL-8 between materials. Moreover, titanium and zirconium oxide group showed lower level of proinflammatory cytokine in peri-implant crevicular fluid (PICF) and also lower plaque assessment score and mucosal tissue condition. These results suggested that gold abutment materials induced more proinflammatory cytokines around dental implant compared with zirconium oxide and titanium.

Implant abutment is considered as the connection between intraosseous and prosthetic part. It is a key zone around dental implant in order to preserve the crestal bone as well as maintain the

peri-implant tissue and adjacent structure. In the oral environment, the surface properties of material involve several conditions in early phase of biofilm formation and maturation. It is important for the selection of the material used in the transmucosal portion along with other physical properties. Various factors affect bacterial adhesion such as surface roughness, surface chemistry, surface free energy, purity, designs and connections.(62, 63) Regarding to plaque retention on abutment surface, a recent literature review showed that surface roughness can affect the biofilm formation and maturation. The rougher surface promoted more cell adhesion and microbial colonization.(62) Various surface roughness parameters were found in different reports. Linear profile (R_a) and surface (S_a) value are the most common used parameters. Bollen and associates concluded that $R_a \leq 0.2$ μm had no or lesser influence of plaque accumulation.(41) A study by Rimondini et al. investigated the proper polishing level of titanium in order to reduce early plaque colonization. Titanium surface with $R_a \leq 0.088$ μm exhibited the lower plaque retention on early 24-hour time period.(42) However, no definite surface roughness values has been introduced as a guideline for plaque deposit. In this study, different materials with extremely low surface roughness (R_a<0.06 μm and S_a<0.08 μm) exhibited different amount of bacterial

adhesion. Gold alloy performed the highest plaque retention under similar roughness. These results were in agreement with previous studies. In vitro study, zirconia, alumina-toughened zirconia, type III gold alloy and cp-titanium with similar roughness were evaluated the initial bacterial adhesion. Gold alloy showed the strongest values for all bacterial strains. Moreover, gold specimen showed the highest polar surface energy and the lowest nonpolar surface energy. They concluded that gold alloy should be used with caution as an abutment material to prevent peri-implantitis.(63) Surface free energy has been proven as one of the factors of material to plaque retention. Previous study investigated the ability of bacterial adhesion to different materials. The results showed that the zirconia material and titanium blasted with zirconia surface exhibited lower surface free energy and lower the adhesion of experimented bacteria compared with polished pure titanium. The surface free energy demonstrated in reducing initial bacterial adhesion to smooth surface.(64) These findings are in contrast to the results of Zhao and colleagues which compared the tissue interaction to bacteria on surface materials of titanium, titanium-zirconium alloy and zirconium oxide. Zirconium oxide appeared with more biofilm formation than titanium and titanium-zirconium alloy because of the roughness of its surface.

They concluded that smooth titanium surface demonstrated suitable for soft tissue seal around implant abutment.(65) In vitro study about the effect of implant abutments on the bacterial profile and biofilm formation showed that titanium disk demonstrated lower biofilm mass and density than zirconium oxide disk. However, type of materials did not affect the bacterial profile around abutment.(66)

Titanium, zirconium oxide and gold alloy are considered the materials of choice for transmucosal implant abutment. Commercially pure titanium demonstrated excellent survival rates and biocompatibility for implant restoration.(3) Over the past few years, the use of zirconium oxide abutment by using CAD-CAM technology has increased because of the trend of esthetic dentistry.(30, 31) Titanium and Zirconium oxide is defined a non-resorbable bioinert metal oxide. Both of them appear an active metal with oxide layer on their surface. While several examinations reported differences in biofilm formation and soft tissue response on titanium and zirconium oxide materials, others demonstrated no difference between the material surfaces.

The application of customized gold alloy known as UCLA abutment has decreased due to the cost of gold alloy. However, this abutment material has its abilities to solve several compromising

cases. Not only surface characteristics but also purity of abutment materials influence early phase of bacterial colonization as well as inflammatory mediators.(58, 62) Gold alloy abutments used in this experiment were fabricated using a yellow gold-based dental casting alloy (Minigold, Ivoclar Vivadent) by technician. The compositions were 59.5%Au, 2.7%Pd, 26.3%Ag, 8.5%Cu and 2.7%Zn. The results of present study pointed to the highest concentration of pro-inflammatory cytokines in gold alloy abutment. These data were correlated with the histological section studies. An animal study by Abrahamsson et al. reported that the mucosal attachment around gold alloy abutment was smaller in dimensions after 6 months of healing.(7) Another animal study revealed that soft tissue recession and bone loss were found in gold alloy abutment while titanium and zirconium oxide were stable at 5-month healing period. Moreover, the connective tissue zone of gold alloy abutment showed amount of fractions of leukocytes than other abutments.(67) Recently, a human study demonstrated that the amount and location of the inflammatory cells with the highest percentage were found in gold alloy group. Titanium and zirconium oxide reported similar mean histological attachment percentages while gold alloy had a significantly lower percentage.(10)

Having created charts (Table 9 and Figure 14) that represented percent of control with two types of measurement for an individual patient in each abutment material group using formula below:

1. Percent of control = $\frac{Present\ Value}{Pilot\ Study\ Value} \times 100\%$
2. Percent of change = $\frac{Present\ Value - Pilot\ Study\ Value}{Pilot\ Study\ Value} \times 100\%$

As the results, trends of how individuals react to abutment become clearer, but these charts did not help us achieve a goal of reducing high standard deviation of the underlying data. Besides the underlying of data cannot be changed from representing different values (percent of control and percent of change), these charts are also hard for person who without statistical background to interpret within a glance.

Although these charts better represented individuals' reactions to different abutment materials, this study aim to compare how abutment materials were different from each other. According to review literatures, these types of charts were not quite in research norms. With these additional drawbacks, the charts should not be included in actual paper because it may be misleading the objectives of this study.

Some might say this study had such a small sample size. Power analysis was performed to determine sample size in order to detect a true effect of this study. Having performed power analysis on pilot study data confirmed with only 12 participants can yield about 95% statistical power. This study ended up having 15 participants with the statistical power of 98% confirmed that this study had enough statistical power to confirm assumptions were made on this study is reliable.

LIMITATIONS AND FURTHER STUDY

Collection of peri-implant crevicular fluid by means of filter paper strips is easy to apply to individual sites with less trauma. However, the disadvantage of this technique is the mean of estimating the volume of sample collected. The capillary tubing or micropipettes and the electronic measuring device (Periotron®) should be used to determine the accurate peri-implant crevicular fluid volume and the sample composition.

The inflammatory response in soft tissue is related to the oral hygiene of patients. Plaque control is the individual ability although the oral hygiene instruction was provided to every participant.

Among implant abutments materials used in this study, the highest fractures were reported for zirconium oxide abutment.(31) In addition to implant surface deterioration at titanium platform, the wear of titanium fixture and zirconium oxide abutment interface have to be concerned.(32) In order to solve these problems, the cement-retained zirconia abutment with titanium base have been introduced into the market.

A further study with longer time points and the inflammatory response of zirconia-coping cemented on titanium-base (Ti-base) abutment should be conducted. A larger sample size is required to determine the effect of abutment materials to production of inflammatory cytokines. Moreover, other mediators should be evaluated in order to understand the biological process respond to materials.

CONCLUSION

Within limitation of this study, gold alloy abutment induced the elevated level of IL-1β and IL-6 in peri-implant crevicular fluid when compared with titanium and zirconium oxide at weeks 4,6 and 8, whereas the expression of IL-8 were observed no significant differences in all time points. Higher plaque score and mucosal tissue index were reported in gold abutment. The implant abutment materials with similar roughness have an influence on the immune response. However, gold alloy abutment has been still used in order to solve the compromising cases. Therefore, strict oral hygiene cares should be given to patients when using gold alloy abutment.

APPENDICES

The average surface roughness of each implant abutment material was summarized in table 10. There were no statistic differences in R_a ($p = 0.3$) and S_a ($p = 0.4$) among dental abutment materials.

Table 10 Mean values of parameters for surface roughness (mean±SD)

Material	Parameter	Mean±SD
Gold alloy	R_a	0.041±0.01
	S_a	0.069±0.037
Titanium	R_a	0.034±0.008
	S_a	0.059±0.036
Zirconium oxide	R_a	0.036±0.005
	S_a	0.0398±0.021

The raw data in this study showed in table 11. Values denoted as NA are non-detected values.

Table 11 Raw data

Week	Material	Patient No.	Age	Gender	Region	Plaque score	Mucosal index	IL-1β	IL-6	IL-8
4	Gold	4	53	F	3	1	1	278.8	NA	204.6
		10	64	M	2	0	1	198.83	NA	204.6
		13	52	F	1	1	1	234.8	3.107	637.3
		17	25	M	1	2	1	133.05	NA	239.3
		24	47	M	4	1	1	287.9	NA	460.8
4	Titanium	6	60	M	4	1	1	126.7	NA	65.81
		8	57	M	3	1	1	21.64	NA	225.2
		12	60	M	3	1	1	14.55	NA	243.3
		14	59	F	3	0	0	33.05	NA	192
		21	51	F	4	1	0	132	NA	395.3
4	Zirconia	1	56	F	2	0	0	82.78	NA	146.7

		5	77	F	4	1	0	33.05	NA	NA
		7	60	M	4	1	1	72.37	NA	NA
		11	64	M	2	0	0	51.56	NA	533.2
		18	59	M	4	0	0	78.4	NA	420.8
6	Gold	4	53	F	3	1	0	257	NA	189
		10	64	M	2	0	1	102.72	NA	4.58
		13	52	F	1	1	1	315.8	15.26	328.9
		17	25	M	1	1	1	148.89	NA	56.68
		24	47	M	4	1	1	185.79	3.44	53.77
6	Titanium	6	60	M	4	1	1	1.19	NA	310.4
		8	57	M	3	0	0	2.21	NA	66.69
		12	60	M	3	1	0	85.79	NA	69.23
		14	59	F	3	1	1	35.34	NA	22.38
		21	51	F	4	1	0	138.6	NA	93.82
6	Zirconia	1	56	F	2	0	0	24.77	NA	NA

		5	77	F	4	0	0	52.87	NA	NA
		7	60	M	4	0	1	44.92	NA	625
		11	64	M	2	0	0	88.13	NA	78.43
		18	59	M	4	0	0	60.88	NA	16.19
8	Gold	4	53	F	3	1	1	167.75	NA	243.3
		10	64	M	2	0	1	269.5	NA	108.2
		13	52	F	1	1	1	123.8	3.845	46.38
		17	25	M	1	1	1	222.1	NA	82.3
		24	47	M	4	1	1	201.6	NA	13.47
8	Titanium	6	60	M	4	1	1	41.15	NA	88.9
		8	57	M	3	1	0	55.03	NA	58.13
		12	60	M	3	0	0	22.65	NA	82.58
		14	59	F	3	1	1	22.65	NA	61.86
		21	51	F	4	1	0	208.4	NA	162.2
8	Zirconia	1	56	F	2	0	0	108.3	NA	156.7

		5	77	F	4	1	1	98.77	NA	52.01
		7	60	M	4	0	0	141.8	NA	75.38
		11	64	M	2	0	0	211.1	NA	94.63
		18	59	M	4	0	0	79.31	NA	44.57
10	Gold	4	53	F	3	1	1	207	0.484	126.3
		10	64	M	2	0	1	286.7	NA	48.76
		13	52	F	1	1	1	319.6	1.35	31.46
		17	25	M	1	1	0	241.6	0.12	70.35
		24	47	M	4	1	1	254.6	NA	8.32
10	Titanium	6	60	M	4	1	0	257.58	0.66	303.6
		8	57	M	3	0	0	88.71	NA	53.48
		12	60	M	3	0	0	244.1	NA	94.36
		14	59	F	3	0	0	352.92	NA	145.8
		21	51	F	4	2	0	177.5	NA	98.99
10	Zirconia	1	56	F	2	0	0	280.52	NA	226.8

		5	77	F	4	1	1	254.11	3.44	248.7
		7	60	M	4	1	0	67.78	NA	73.99
		11	64	M	2	0	0	256.79	NA	106.6
		18	59	M	4	0	0	272.06	NA	106.5

REFERENCES

1. Lekholm UL, Gunne J, Henry P, Higuchi K, Lindén U, Bergstrom C, et al. Survival of the Brånemark Implant in Partially Edentulous Jaws: A 10-Year Prospective Multicenter Study. International journal of oral and maxillofacial implants. 1999;14(5):639-45.

2. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. Periodontology. 2000;17:63-76.

3. Rompen E, Domken O, Degidi M, Farias Pontes AE, A P. The effect of material characteristics, of surface topography and of implant components and connections on soft tissue integration : a literature review. Clinical oral implants research. 2006;17(Suppl. 2):55-67.

4. Glauser R, Sailer I, Wohlwend A, Studer S, Schibli M, Schärer P. Experimental Zirconia Abutments for Implant-Supported Single-Tooth Restorations in Esthetically Demanding Regions- 4-Year Results of a Prospective Clinical Study. International journal of prosthodontics. 2004;17(3):285-90.

5. Linkevicius T AP. Influence of abutment material on stability of peri-implant tissues International journal of oral & maxillofacial implants. 2008;23(3):449-56.

6. Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J. Morphogenesis of the peri-implant mucosa: an experimental study in dogs. Clinical oral implants research. 2007;18(1):1-8.

7. Abrahamsson I. BT, Glantz P-O., J. Lindhe. The mucosal attachment at different abutments. An experimental study in dogs. J Clin Periodontol. 1998;25:721-7.

8. Kawahara H, Kawahara D, Mimura Y, Takashima Y, JL O. Morphologic studies on the biologic seal of titanium dental implants. report II. In vivo study on the defending mechanism of epithelial adhesion: attachment against invasive factors. International journal of oral and maxillofacial implants. 1998;13(4):465-73.

9. Schroeder HE, Listgarten MA. The gingival tissues: the architecture of periodontal protection. Periodontology 2000. 1997;13(1):91-120.

10. Sampatanukul T, Serichetaphongse P, Pimkhaokham A. Histological evaluations and inflammatory responses of different dental implant abutment materials: A human histology pilot study. Clinical implant dentistry and related research. 2017;20(2):160-9.

11. Moon IS, Berglundh T, Abrahamsson I, Linder E, Lindhe J. The barrier between the

keratinized mucosa and the dental implant: an experimental study in the dog. Journal of clinical periodontology. 1999;26(10):658-63.

12. Abrahamsson I, Cardaropoli G. Peri-implant hard and soft tissue integration to dental implants made of titanium and gold. Clinical oral implants research. 2007;18(3):269-74.

13. Duarte PM, Serrao CR, Miranda TS, Zanatta LC, Bastos MF, Faveri M, et al. Could cytokine levels in the peri-implant crevicular fluid be used to distinguish between healthy implants and implants with peri-implantitis? A systematic review. Journal of periodontal research. 2016;51(6):689-98.

14. Barwacz CA, Brogden KA, Stanford CM, Dawson DV, Recker EN, Blanchette D. Comparison of pro-inflammatory cytokines and bone metabolism mediators around titanium and zirconia dental implant abutments following a minimum of 6 months of clinical function. Clinical oral implants research. 2015;26(4):e35-41.

15. Adell R, Lekholm U, Rockler BR, PI B. A I5-year study of osseointegrated implants in the treatment of the edentulous jaw. International journal of oral surgery. 1981;10(6):387-416.

16. Moraschini V, Poubel LA, Ferreira VF, Barboza Edos S. Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. International journal of oral and maxillofacial surgery. 2015;44(3):377-88.

17. Albrektsson T. A multicenter report on osseointegrated oral implants. Journal of Prosthetic Dentistry. 1998;60(1):75-84.

18. Anton Sculean RG, Dieter D. Bosshardt. Soft tissue wound healingaround teeth and dental implants. J Clin Periodontol. 2014;41(Suppl. 15):S6–S22.

19. Chandki R, Kala M. Natural tooth versus implant: a key to treatment planning. Journal of oral implantology. 2012;38(1):95-100.

20. Vigolo P, Givani A, Majzoub Z, Cordioli G. A 4-year prospective study to assess peri-implant hard and soft tissues adjacent to titanium versus gold-alloy abutments in cemented single implant crowns. Journal of Prosthodontics. 2006;15(4):250-6.

21. Roach P, Eglin D, Rohde K, Perry CC. Modern biomaterials: a review - bulk properties and implications of surface modifications. Journal of materials science: materials in medicine. 2007;18(7):1263-77.

22. van Brakel R, Meijer GJ, Verhoeven JW, Jansen J, de Putter C, Cune MS. Soft tissue

response to zirconia and titanium implant abutments: an in vivo within-subject comparison. Journal of clinical periodontology. 2012;39(10):995-1001.

23. Lewis S, Beumer J, Perri G, Hornburg W. Single tooth implant supported restorations. International journal of oral and maxillofacial implants. 1988;3(1):25-30.

24. Lewis S, Beumer J, Hornburg W, Moy P. The "UCLA" abutment. International journal of oral & maxillofacial implants. 1988;3(3):183-9.

25. Linkevicius T, Vaitelis J. The effect of zirconia or titanium as abutment material on soft peri-implant tissues: a systematic review and meta-analysis. Clinical oral implants research. 2015;26 Suppl 11:139-47.

26. de Avila ED, Avila-Campos MJ, Vergani CE, Spolidorio DM, de Assis Mollo Jr F. Structural and quantitative analysis of a mature anaerobic biofilm on different implant abutment surfaces. The Journal of prosthetic dentistry. 2016;115(4):428-36.

27. Ismail F, Eisenburger M, Grade S, Stiesch M. In situ biofilm formation on titanium, gold alloy and zirconia abutment materials. Dentistry. 2016;6(11):1-5.

28. Sailer I, Zembic A, Jung RE, Siegenthaler D, Holderegger C, Hammerle CH. Randomized controlled clinical trial of customized zirconia and titanium implant abutments for canine and posterior single-tooth implant reconstructions: preliminary results at 1 year of function. Clinical oral implants research. 2009;20(3):219-25.

29. Zembic A, Sailer I, Jung RE, Hammerle CH. Randomized-controlled clinical trial of customized zirconia and titanium implant abutments for single-tooth implants in canine and posterior regions: 3-year results. Clinical oral implants research. 2009;20(8):802-8.

30. Lops D, Bressan E, Chiapasco M, Rossi A, Romeo E. Zirconia and titanium implant abutments for single-tooth implant prostheses after 5 years of function in posterior regions. International journal of oral & maxillofacial implants. 2013;28(1):281-7.

31. Bidra AS, Rungruanganunt P. Clinical outcomes of implant abutments in the anterior region: a systematic review. Journal of Esthetic and Restorative Dentistry. 2013;25(3):159-76.

32. M. Pettersson, P. Kelk, G. N. Belibasakis, D. Bylund, M. Molin Thore n, Johansson A. Titanium ions form particles that activate and execute interleukin-1b release from lipopolysaccharide-primed macrophages. Journal of Clinical Periodontology. 2017;52(1):21–32.

33. Bostanci N, Belibasakis GN. Gingival crevicular fluid and its immune mediators in the

proteomic era. Periodontology 2000. 2018;76:68-84.

34. Griffiths, Gareth S. Formation, collection and significance of gingival crevice fluid. Periodontology 2000. 2003;31(1):32-42.

35. Attstrom R, Egelberg J. Emigration of blood neutrophils and monocytes into the gingivai crevices. Journal of periodontal research. 1970;5(1):48-55.

36. Delima AJ, Van Dyke TE. Origin and function of the cellular components in gingival crevice fluid. Periodontology 2000. 2003;31:55-76.

37. PetkoviĆ AB, MatiĆ SM, DV V, StamatoviĆ NV, TodoroviĆ TM. Cytokines in pathogenesis of peri-implantitis. Vojnosanitetski pregled. 2011;68(5):435-40.

38. Stadler AF, Angst PD, Arce RM, Gomes SC, Oppermann RV, Susin C. Gingival crevicular fluid levels of cytokines/chemokines in chronic periodontitis: a meta-analysis. Journal of clinical periodontology. 2016;43(9):727-45.

39. VJ. U. Gingival crevice fluid–an introduction. Periodontology 2000. 2003;31:9-11.

40. Lee A, Wang HL. Biofilm related to dental implants. Implant dentistry. 2010;19(5):387-93.

41. Bollenl CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention - A review of the literature. Dental materials. 1997;13(4):258–69.

42. Rimondini L, Farè S, Brambilla E, Felloni A, Consonni C, Brossa F, et al. The effect of surface roughness on early in vivo plaque colonization on titanium. Journal of periodontology. 1997;68(6):556-62.

43. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. Roitt's Essential Immunology. 13th ed. Chichester, West Sussex: John Wiley & Sons, Ltd; 2017. 576 p.

44. Kao RT, Curtis DA, Richards DW, Preble J. Increased interleukin-1 β in the crevicular fluid of diseased implants. International journal of oral & maxillofacial implants. 1995:696-701.

45. Masashi M., Jun-ichi T., Yumi K., Satoru S., Yasuko N., Yukinao K., et al. Osteocalcin, deoxypyridinoline and interleukin-1 β in peri-implant crevicular fluid of patients with peri-implantitis. Clinical oral implants research. 2002;13:637-43.

46. Ataoglu H, Alptekin NO, Haliloglu S, Gursel M, Ataoglu T, Serpek B, et al. Interleukin-1beta, tumor necrosis factor-alpha levels and neutrophil elastase activity in peri-implant crevicular fluid. Clinical oral implants research. 2002;13(5):470-6.

47. Negahdari R, Rahbar M, Fakhrzadeh V, Eslami H, Akbari T, Bohluli S. Comparison of proinflammatory cytokine levels in gingival crevicular fluid around dental implants with ceramic and titanium abutments. The journal of contemporary dental practice. 2017;18(9):831-6.

48. Zhang Y, Wang C, Jinbu Y, Itoh H, Kusama M. Increased IL-6 levels in Peri-implant crevicular fluid correlate with peri-implantitis. Oral Medicine & Pathology. 2005:95-9.

49. Severino VO, Napimoga MH, de Lima Pereira SA. Expression of IL-6, IL-10, IL-17 and IL-8 in the peri-implant crevicular fluid of patients with peri-implantitis. Archives of oral biology. 2011;56(8):823-8.

50. Yaghobee S, Khorsand A, Rasouli Ghohroudi AA, Sanjari K, Kadkhodazadeh M. Assessment of interleukin-1beta and interleukin-6 in the crevicular fluid around healthy implants, implants with peri-implantitis, and healthy teeth: a cross-sectional study. Journal of the Korean Association of Oral and Maxillofacial Surgeons. 2014;40(5):220-4.

51. Nogueira-Filho G, Pesun I, Isaak-Ploegman C, Wijegunasinghe M, Wierzbicki T, McCulloch CA. Longitudinal comparison of cytokines in peri-implant fluid and gingival crevicular fluid in healthy mouths. Journal of periodontology. 2014;85(11):1582-8.

52. Cionca N, Hashim D, Cancela J, Giannopoulou C, Mombelli A. Pro-inflammatory cytokines at zirconia implants and teeth. A cross-sectional assessment. Clinical oral investigations. 2016;20(8):2285-91.

53. Angus W. Thomson, Lotze MT. The Cytokine Handbook. 4th ed: Elsevier Science; 2003. 1572 p.

54. Abul Abbas, Andrew H. H. Lichtman, Pillai S. Basic Immunology: Functions and Disorders of the Immune System. 4th ed: Elsevier; 2012. 336 p.

55. Lindquist LW, Rocker B, Carlsson GE. Bone resorption around fixures in edentulous patients treated with mandibular fixed tissue-intregated prostheses. The Journal of prosthetic dentistry. 1988;59:59–63.

56. Apse P ZG, Schmitt A, Lewis DW. The longitudinal effectiveness of osseointegrated dental implants. The Toronto study: periimplant mucosal response. International journal of Periodontics & Restorative Dentistry. 1991;11:95-111.

57. Gurkan A, Tekdal GP, Bostanci N, Belibasakis GN. Cytokine, chemokine, and growth factor levels in peri-implant sulcus during wound healing and osseointegration after piezosurgical

versus conventional implant site preparation: Randomized, controlled, split-mouth trial. Journal of periodontology. 2018:1-11.

58. Amalia Machado Bielemann, Raissa Micaella Marcello-Machado, Altair Antoninha Del Bel Cury, Faot F. Systematic review of wound healing biomarkers in peri-implant crevicular fluid during osseointegration. 2018.

59. Petkovi$\acute{C}$ AB, Mati$\acute{C}$ SM, Stamatovi$\acute{C}$ NV, Vojvodi$\acute{C}$ DV, Todorovi$\acute{C}$ TM, Lazi$\acute{C}$ ZR, et al. Proinflammatory cytokines (IL-1 β and TNF- α) and chemokines (IL-8 and MIP-1 α) as markers of peri-implant tissue condition. International journal of oral and maxillofacial surgery. 2010;39(5):478-85.

60. Ghassib I, Chen Z, Zhu J, Wang HL. Use of IL-1 beta, IL-6, TNF-alpha, and MMP-8 biomarkers to distinguish peri-implant diseases: A systematic review and meta-analysis. Clinical implant dentistry and related research. 2019;21(1):190-207.

61. Liu J, Li R, Liu T, Rausch-Fan X, Wang M. High mobility group box 1 protein level as a novel biomarker for the development of peri-Implant disease. Scientific reports. 2017;7(1):7027.

62. Han A, Tsoi JKH, Rodrigues FP, Leprince JG, Palin WM. Bacterial adhesion mechanisms on dental implant surfaces and the influencing factors. International journal of adhesion and adhesives. 2016;69:58-71.

63. Kim HY, Yeo IS, Lee JB, Kim SH, Kim DJ, Han JS. Initial in vitro bacterial adhesion on dental restorative materials. The International journal of artificial organs. 2012;35(10):773-79.

64. Al-Radha AS, Dymock D, Younes C, O'Sullivan D. Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion. Journal of dentistry. 2012;40(2):146-53.

65. Zhao B, van der Mei HC, Subbiahdoss G, de Vries J, Rustema-Abbing M, Kuijer R, et al. Soft tissue integration versus early biofilm formation on different dental implant materials. Dental materials. 2014;30(7):716-27.

66. de Avila ED, Vergani CE, Mollo Junior FA, Junior MJ, Shi W, Lux R. Effect of titanium and zirconia dental implant abutments on a cultivable polymicrobial saliva community. The Journal of prosthetic dentistry. 2017;118(4):481-7.

67. Welander M, Abrahamsson I, Berglundh T. The mucosal barrier at implant abutments of different materials. Clinical oral implants research. 2008;19(7):635-41.

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